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WO 2004/045511 A2

(54) Title: NON-STEROIDAL FARNESOID X RECEPTOR MODULATORS

(57) Abstract: The efficient regulation of cholesterol synthesis, metabolism, acquisition, and transport is an essential component of lipid homeostasis. The farnesoid X receptor (FXR) is a transcriptional sensor for bile acids, the primary product of cholesterol metabolism. Accordingly, the development of potent, selective, small molecule agonists, partial agonists, and antagonists of FXR would be an important step in further deconvoluting FXR physiology. In accordance with the present invention, the identification of novel potent FXR activators is described. Two derivatives of invention compounds, bearing stilbene or biaryl moieties, contain members that are the most potent FXR agonists reported to date in cell-based assays. These compounds are useful as chemical tools to further define the physiological role of FXR as well as therapeutic leads for the treatment of diseases linked to cholesterol, bile acids and their metabolism and homeostasis.

NON-STEROIDAL FARNESOID X RECEPTOR MODULATORS

FIELD OF THE INVENTION

[0001] The present invention relates to new chemical entities. In a particular aspect, the present invention relates to non-steroidal modulators of farnesoid X receptors (FXR). In another aspect, the present invention relates to methods for modulating FXR-mediated processes employing the novel compounds described herein.

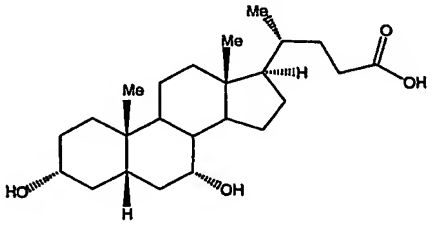
BACKGROUND OF THE INVENTION

[0002] The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

[0003] The efficient regulation of cholesterol biosynthesis, metabolism, acquisition and transport is an essential function of mammalian cells. High levels of cholesterol are associated with atherosclerosis, a leading cause of death in the western world and a major risk factor correlated with the occurrence of coronary heart disease and stroke. Until recently, recommendations for the treatment of hypercholestemia were focused on the use of statins, which inhibit the de novo biosynthesis of cholesterol, and the use of bile acid sequestering agents. While statin-based agents are still in widespread use as cholesterol-lowering drugs, an evolving understanding of the mechanisms controlling cholesterol homeostasis has led to new molecular targets as candidates in therapeutic intervention.

[0004] Cholesterol metabolism is controlled through a complex feedback loop involving cholesterol itself and bile acids (which are primary oxidation products), and through secretion in the gut, the single most critical regulators of cholesterol absorption. The nuclear receptors LXR (liver X receptor) and FXR (farnesoid X receptor) are the specialized sensors of cholesterol and bile acids that control transcription of networks encoding key metabolic enzymes. For example activation of LXR by oxysterols (i.e., mono-oxygenated cholesterol metabolites) leads to the up-regulation of CYP7A1, the enzyme that catalyzes the rate limiting step in the conversion of cholesterol to bile acids. In turn, bile acids such as chenodeoxycholic acid (CDCA, **1**, a low affinity endogenous agonist for FXR, whose

structure is shown below) are potent ligands for FXR, whose activation leads to down-regulation of CYP7A1, leading to the completion of the feedback circuit.



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In this circuit FXR induces the expression of a transcriptional repressor SHP (small heterodimer partner) which in turn binds to LRH-1 (liver receptor homolog), which is required in CYP7A activation. Additionally, both LXR and FXR are implicated in the regulation of several other gene products involved in cholesterol absorption, metabolism and transport.

[0005] Thus, the identification of potent, selective, small molecule FXR agonists, partial agonists and antagonists would be powerful tools and would have many potential applications. For example, such compounds would facilitate the *in vivo* analysis of FXR physiology *in vivo*. In addition, such compounds, in conjunction with DNA arraying technology, might allow for the discovery of new gene products under the control of FXR. Further, FXR modulators might find potential utility in the treatment of cholestasis and other disease states associated with aberrant levels, flow and release of bile acids. Moreover, in the absence of a crystal structure of FXR, a thorough structure-activity relationship (SAR) study of ligands that modulate the activity of FXR would allow for the delineation of the structural requirements for ligand binding and might aid in the design of future ligands and potential therapeutics.

SUMMARY OF THE INVENTION

[0006] In accordance with the present invention, the identification of novel potent FXR activators is described. Initial screening of a 10,000-membered, diversity-orientated library of benzopyran containing small molecules for FXR activation utilizing a cell-based reporter assay led to the identification of several lead compounds owning low micromolar activity (EC_{50}'s = 5 – 10 μM). These compounds were systematically modified employing parallel

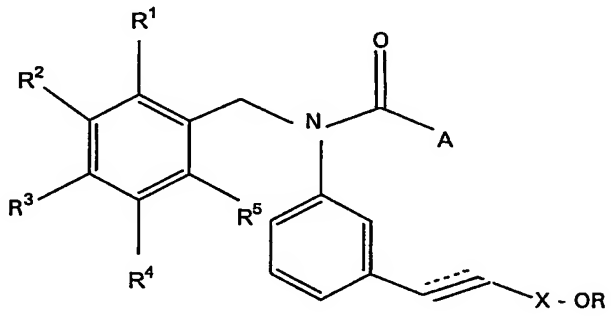
solution-phase synthesis and solid-phase synthesis to provide numerous compounds that potently activate FXR. Two derivatives of invention compounds, bearing stilbene or biaryl moieties, contain members that are the most potent FXR agonists reported to date in cell-based assays. These compounds are useful as chemical tools to further define the physiological role of FXR as well as therapeutic leads for the treatment of diseases linked to cholesterol, bile acids and their metabolism and homeostasis.

BRIEF DESCRIPTION OF THE FIGURE

[0007] Figure 1 summarizes the efficacy of the functional assay for the identification of FXR agonists, using the known FXR agonist, chenodeoxycholic acid (CDCA).

DETAILED DESCRIPTION OF THE INVENTION

[0008] In accordance with the present invention, there are provided compounds having the structure:



wherein:

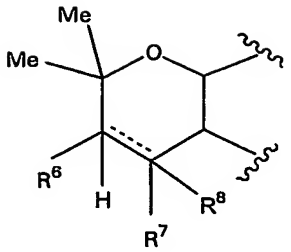
A is a C3 up to C8 branched chain alkyl or substituted alkyl group, a C3 up to C7 cycloalkyl or substituted cycloalkyl, an optionally substituted aryl or an optionally substituted heteroaryl,

X is –C(O)- or $-CH_2-$,

R is methyl or ethyl,

R^1 is H, hydroxy, alkoxy, benzoyloxy, mesityloxy, or $-OCH_2C(O)OC_2H_5$,

R^2 is H or R^2 can cooperate with R^3 to form a benzopyran, wherein the pyran ring has the structure:



wherein:

R^6 is not present if the pyran ring is unsaturated, or, if present, is selected from H, -OR, wherein R is alkyl or acyl, or R^6 can cooperate with R^7 to form a cyclic acetal, a cyclic ketal, or a cyclopropyl moiety, and

only one of R^7 and R^8 is present if the pyran ring is unsaturated, or R^7 and R^8 are independently H, carboxyl, cyano, hydroxy, alkoxy, thioalkyl, aryl, or R^7 and R^8 taken together comprise a carbonyl oxygen or an oxime nitrogen, or either R^7 or R^8 can cooperate with R^6 to form a cyclic acetal, a cyclic ketal, or a cyclopropyl moiety,

R^3 can cooperate with R^2 to form a benzopyran having the structure set forth above, or R^3 is alkenyl, optionally substituted aryl or heteroaryl, or optionally substituted arylalkenyl or heteroarylalkenyl,

R^4 is H or hydroxy, and

R^5 is H, hydroxy, alkoxy or aryloxy.

[0009] As employed herein, "alkyl" refers to saturated straight or branched chain hydrocarbon radical having in the range of 1 up to about 20 carbon atoms. "Lower alkyl" refers to alkyl groups having in the range of 1 up to about 5 carbon atoms. "Substituted alkyl" refers to alkyl groups further bearing one or more substituents selected from hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), cycloalkyl, substituted cycloalkyl, heterocyclic, substituted heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl, aryloxy, substituted aryloxy, halogen, trifluoromethyl, cyano, nitro, nitrone, amino, amido, —C(O)H, acyl, oxyacyl, carboxyl, carbamate, dithiocarbamoyl, sulfonyl, sulfonamide, sulfuryl, and the like.

[0010] As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 20 carbon atoms, and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

[0011] As employed herein, "alkoxy" refers to –O-alkyl groups having in the range of 2 up to 20 carbon atoms and "substituted alkoxy" refers to alkoxy groups further bearing one or more substituents as set forth above.

[0012] As employed herein, "cycloalkyl" refers to a cyclic ring-containing groups containing in the range of about 3 up to about 8 carbon atoms, and "substituted cycloalkyl" refers to cycloalkyl groups further bearing one or more substituents as set forth above.

[0013] As employed herein, "heterocyclic" refers to cyclic (i.e., ring-containing) groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 3 up to 14 carbon atoms and "substituted heterocyclic" refers to heterocyclic groups further bearing one or more substituents as set forth above.

[0014] As employed herein, "aryl" refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.

[0015] As employed herein, "aryloxy" refers to –O-aryl groups having in the range of 6 up to 14 carbon atoms and "substituted aryloxy" refers to aryloxy groups further bearing one or more substituents as set forth above.

[0016] As employed herein, "arylalkenyl" refers to aryl-substituted alkenyl groups and "substituted arylalkenyl" refers to arylalkenyl groups further bearing one or more substituents as set forth above.

[0017] As employed herein, "heteroaryl" refers to aromatic groups having in the range of 4 up to about 13 carbon atoms, and at least one heteroatom selected from O, N, S, or the like;

and "substituted heteroaryl" refers to heteroaryl groups further bearing one or more substituents as set forth above.

[0018] As employed herein, "heteroarylalkenyl" refers to heteroaryl-substituted alkenyl groups and "substituted heteroarylalkenyl" refers to heteroarylalkenyl groups further bearing one or more substituents as set forth above.

[0019] As employed herein, "acyl" refers to alkyl-carbonyl species.

[0020] As employed herein, "halogen" refers to fluoride, chloride, bromide or iodide atoms.

[0021] As employed herein, reference to "a carbamate group" embraces substituents of the structure $-O-C(O)-NR_2$, wherein each R is independently H, alkyl, substituted alkyl, aryl or substituted aryl as set forth above.

[0022] As employed herein, reference to "a dithiocarbamate group" embraces substituents of the structure $-S-C(S)-NR_2$, wherein each R is independently H, alkyl, substituted alkyl, aryl or substituted aryl as set forth above.

[0023] As employed herein, reference to "a sulfonamide group" embraces substituents of the structure $-S(O)_2-NH_2$.

[0024] As employed herein, "sulfuryl" refers to substituents of the structure $=S(O)_2$.

[0025] As employed herein, "amino" refers to the substituent $-NH_2$.

[0026] As employed herein, "monoalkylamino" refers to a substituent of the structure –NHR, wherein R is alkyl or substituted alkyl as set forth above.

[0027] As employed herein, "dialkylamino" refers to a substituent of the structure $-NR_2$, wherein each R is independently alkyl or substituted alkyl as set forth above.

[0028] As employed herein, reference to "an amide group" embraces substituents of the structure $-C(O)-NR_2$, wherein each R is independently H, alkyl, substituted alkyl, aryl or substituted aryl as set forth above. When each R is H, the substituent is also referred to as "carbamoyl" (i.e., a substituent having the structure $-C(O)-NH_2$). When only one of the R groups is H, the substituent is also referred to as "monoalkylcarbamoyl" (i.e., a substituent having the structure -C(O)-NHR, wherein R is alkyl or substituted alkyl as set forth above) or "arylcarbamoyl" (i.e., a substituent having the structure -C(O)-NH(aryl), wherein aryl is as defined above, including substituted aryl). When neither of the R groups are H, the substituent is also referred to as "di-alkylcarbamoyl" (i.e., a substituent having the structure $-C(O)-NR_2$, wherein each R is independently alkyl or substituted alkyl as set forth above).

[0029] In accordance with a particular embodiment of the present invention, presently preferred compounds are those wherein A is a C5-C7 cycloalkyl group.

[0030] In accordance with another particular embodiment of the present invention, presently preferred compounds are those wherein X is –C(O)-.

[0031] In accordance with yet another particular embodiment of the present invention, presently preferred compounds are those wherein R^1 is hydrogen.

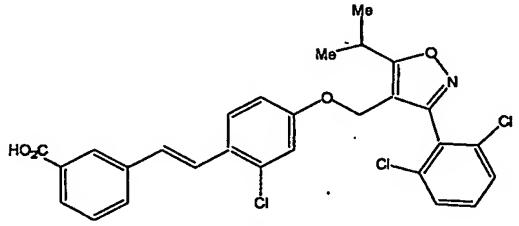
[0032] In accordance with still another particular embodiment of the present invention, presently preferred compounds are those wherein R^2 and R^3 cooperate to form a benzopyran.

[0033] In accordance with a further particular embodiment of the present invention, presently preferred compounds are those wherein R^3 is alkenyl, thereby producing a cinnamate derivative.

[0034] In accordance with a still further embodiment of the present invention, presently preferred compounds are those wherein R^3 is an optionally substituted aryl or heteroaryl moiety, thereby producing biphenyl derivatives.

[0035] In accordance with yet another embodiment of the present invention, presently preferred compounds are those wherein R^3 is an optionally substituted arylalkenyl or heteroarylalkenyl moiety, thereby producing stilbene derivatives.

[0036] As there was, prior to the present invention, only one example of a high affinity, non-steroidal agonist for FXR, i.e., GW 4064 (**3**, having an EC_{50} = 80 nM, structure shown below), the strategy adopted herein for identification of additional potent compounds involved screening a 10,000-membered library constructed around the privileged 2,2-dimethylbenzopyran scaffold.



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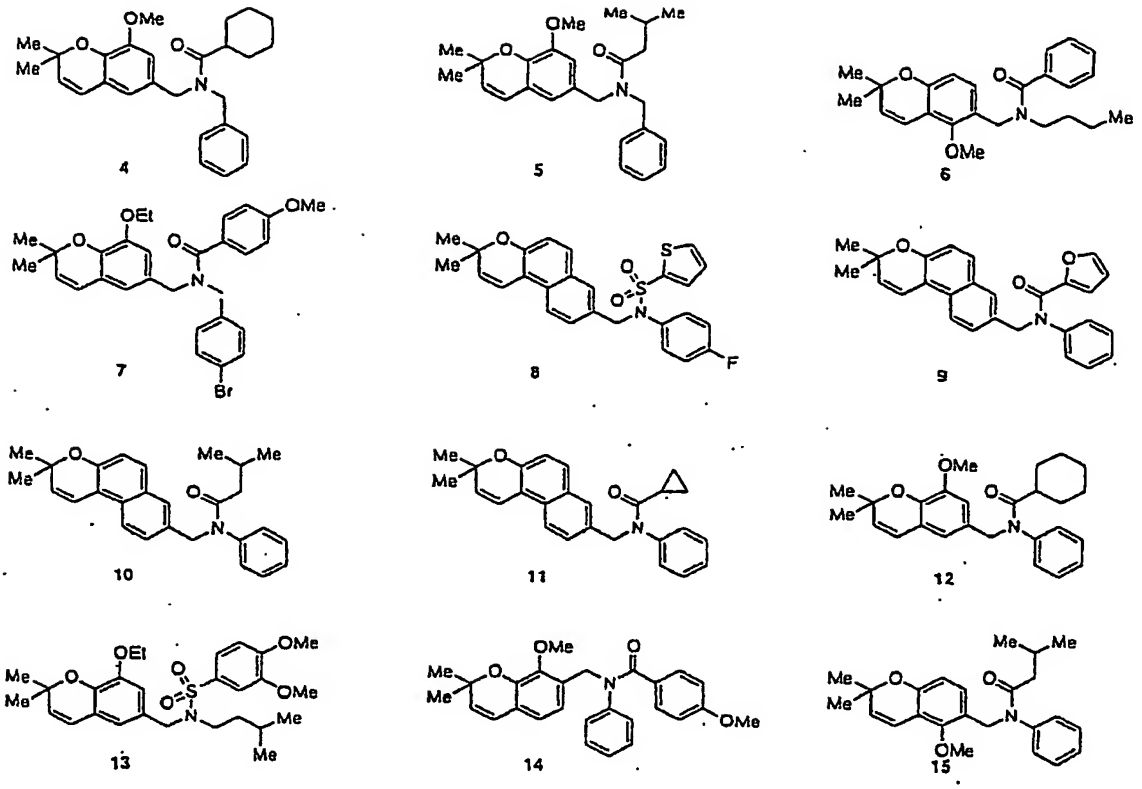
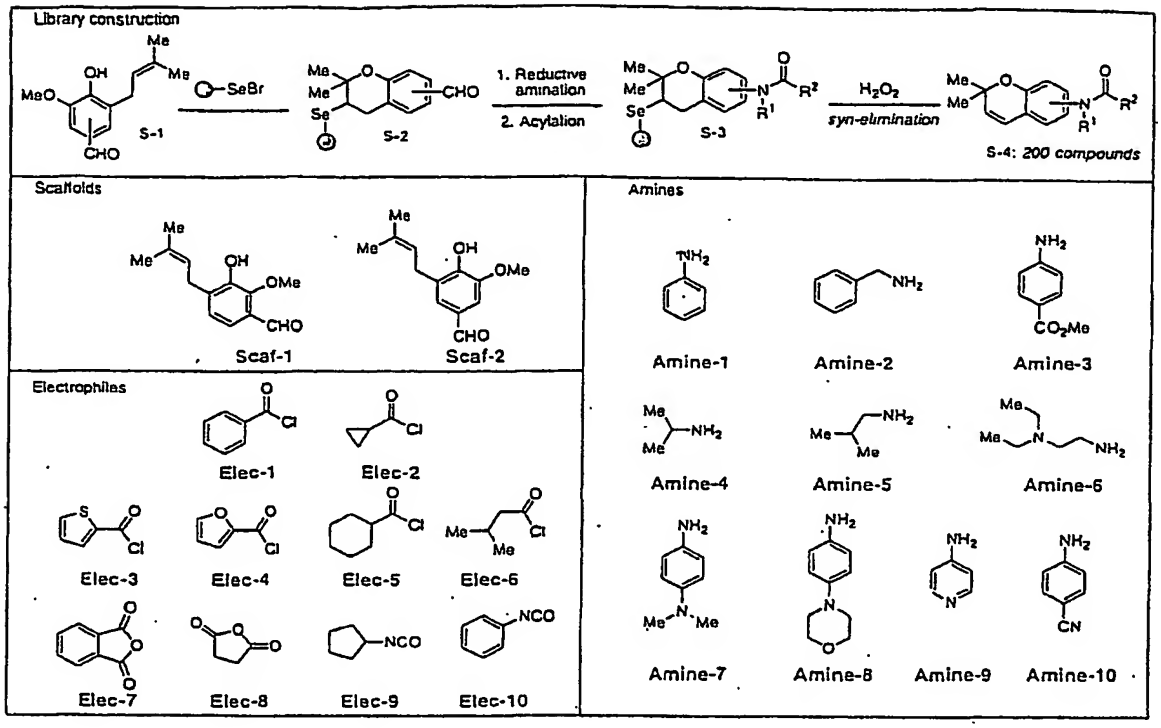
Such privileged structures are attractive starting points for lead compound discovery, particularly when there exists little structural information regarding the target, as they show good binding affinity toward a wide variety of enzymes and receptors. The initial hits discovered from screening of this library for FXR activation could be further modified for enhanced potency and pharmacological properties suitable for the applications mentioned above. Implementation of such a strategy is described herein, culminating in the discovery of numerous potent and selective activators of FXR.

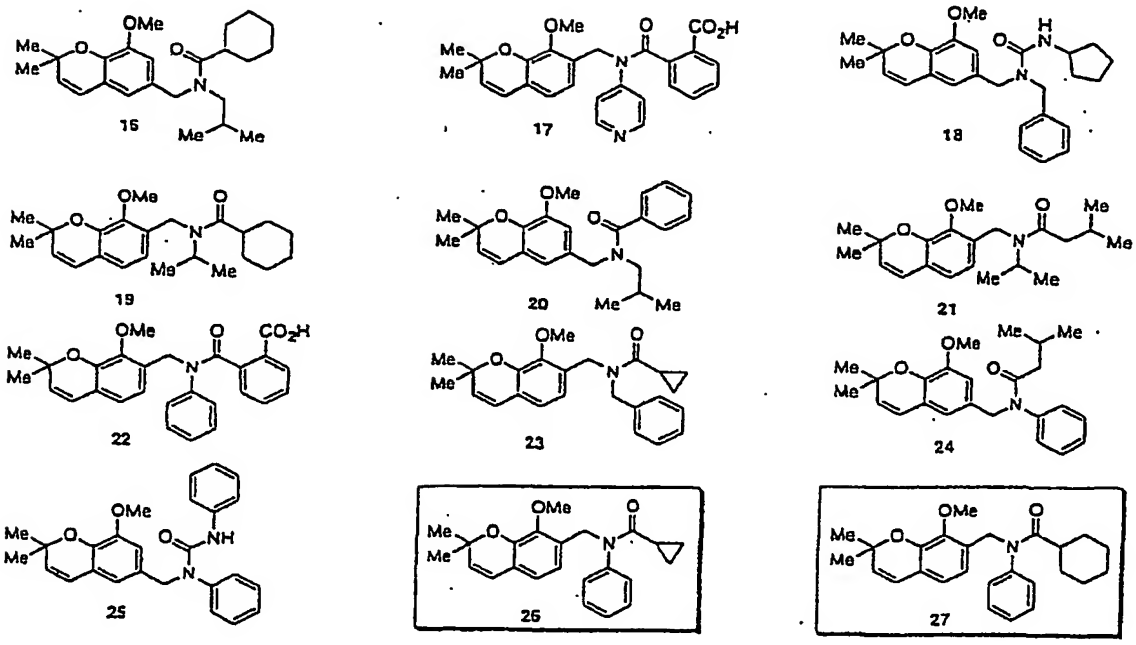
[0037] Thus, in accordance with the present invention, a cell-based transcription assay is employed in which an FXR responsive promoter is linked to a luciferase reporter as the primary screen (see Example 1). In addition to ensuring that only cell permeable compounds were selected for further optimization, this approach allows for the detection of FXR activation in a natural system (i.e., correct folding of the protein and in the presence of a complete compliment of co-activators and co-repressors). Initial screening of a 10,000-membered combinatorial library of benzopyran-based small molecules in this high-throughput, cell-based assay for FXR activation produced several lead compounds, **4 – 15**, whose structures are shown below:



[0038] Guided by the preliminary structure-activity relationships (SAR) gained from the evaluation of this initial library, a follow-up focused library of about 200 benzopyran-based compounds was designed and synthesized on solid support employing the protocol set forth below.

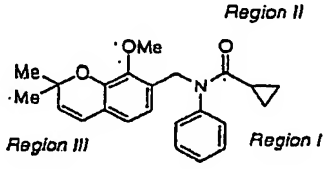


[0039] A selection of the most active compounds, possessing activities from 5 – 10 μM, discovered from this second round of screening, includes compounds **16-27**, as shown below:



[0040] Compounds **26** and **27** proved to be among the most active at this stage and were the subject of further modification as described below.

[0041] With initial lead compounds identified and validated, the stage is set for the systematic modification of the three regions of lead compound **26**, as shown below:

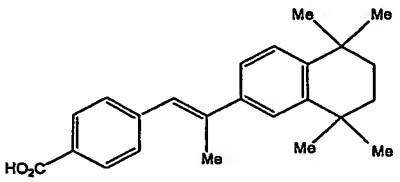


26

[0042] As detailed in the following sections, focused libraries were synthesized and screened in the cell-based assay in order to evaluate the structural requirements of each region of the molecule for potent FXR agonism. At this point parallel solution-phase chemistry was selected for the construction of additional focused libraries. This shift away

from solid-phase chemistry provided maximum flexibility in efforts to rapidly and systematically modify each region of the lead molecules using smaller designed libraries.

[0043] Most of the FXR agonists reported to date, including CDCA (**1**; see structure above), TTNPB (**2**; structure shown below) and GW4064 (**3**; see structure above), contain a carboxylic acid moiety.



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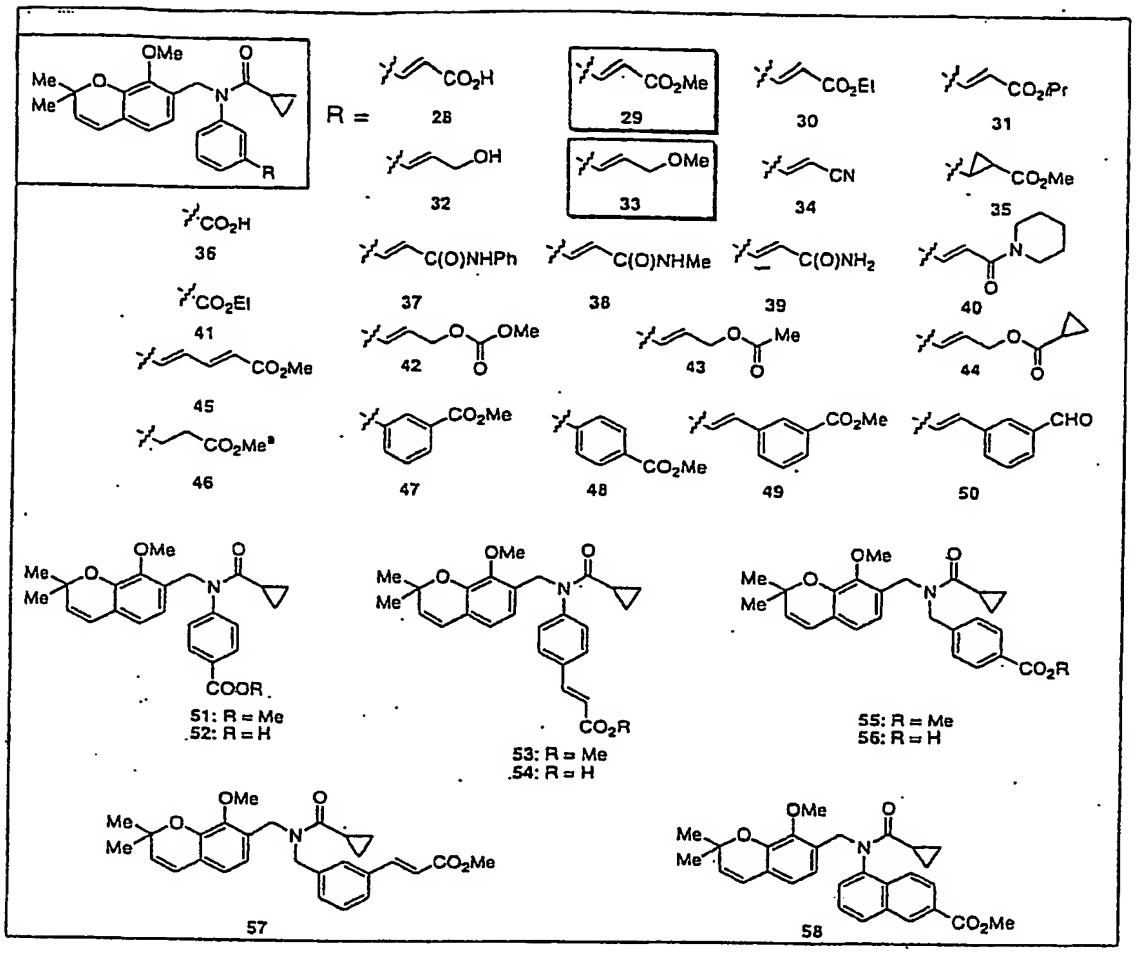
It was reasoned, therefore, that incorporation of an acid unit within either region I, II or III of lead compound **26** (as illustrated above) would confer increased potency upon this rather weak ligand (5 – 10 μM) identified via HTS.

Evaluation Of The Benzopyran Region I SAR

[0044] Guided by this reasoning, the SAR of region I was evaluated. Several compounds displaying the acid unit in various positions (e.g., Compounds **28**, **36**, **52**, **54** and **56**), were prepared (see, Examples 3 to 6) and tested.



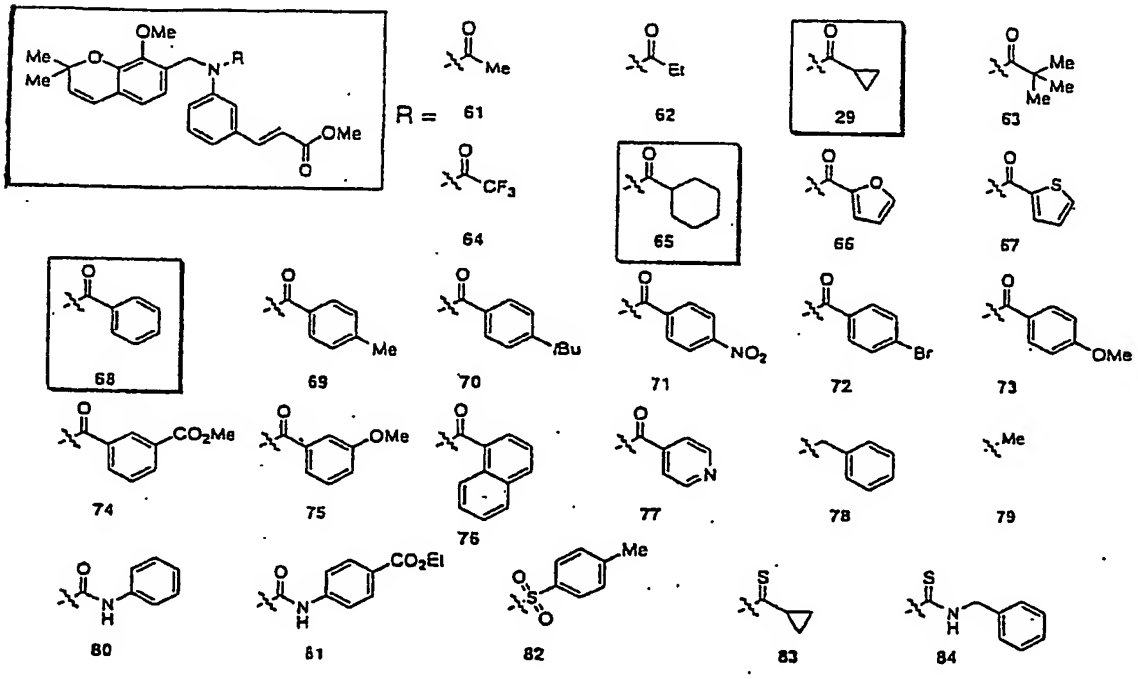
[0045] None of these compounds, however, showed improved activation of FXR. Interestingly, compound **29**, bearing a meta methyl acrylate moiety, was a substantially better activator of FXR than compound **26**.

[0046] In further refining the SAR of region I, it was observed that the location of the methyl acrylate moiety at the meta position was beneficial to achieve potent activation of FXR, as compound **53**, bearing a para methyl acrylate, does not activate FXR under the conditions tested. In order to further examine what functionality was tolerable at the meta position, additional compounds with meta substituents (as shown above) were synthesized. From biological screening of these compounds it became clear that the length and rigidity of

the tether between the aromatic core and the interacting functionality (either methyl ester or methyl ether) are important for FXR agonism. For instance, compounds **41** and **45** appear to possess either too short or too long of a tether for potent activity; compounds **35** and **46 – 49** presumably cannot adopt the correct orientation for potent activation; and compounds **30, 31, 34, 38, 39, 40** and **50** do not apparently present the correct interacting functionality to the receptor as they are inactive. Indeed, of all the analogs designed to probe the SAR of region I, only compounds **29** and **33** are capable of activating FXR to a significant extent. Due to relative ease of synthesis of compound **29** this analog was chosen as a starting point for the modification of region II.

Evaluation of The Benzopyran Region II SAR

[0047] Benzopyran region II SAR was evaluated through traditional solution phase chemistry to see the effect of various substitution patterns in this region of the molecule. Thus, compounds **61-84** (structures shown below) were prepared (see, Example 7) and tested.

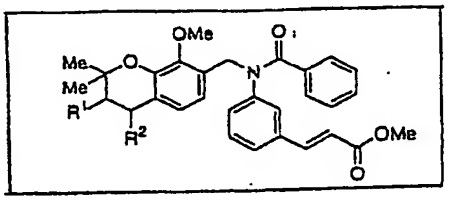


[0048] Only compounds **65** (EC_{50} = 358 nM) and **68** (EC_{50} = 1,000 nM) were more effective than compound **29** in activating FXR. Substituted aromatic amide derivatives such

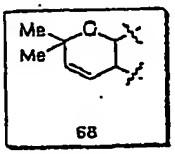
as 69 – 77 were all found to be less active than the parent compound 68. Alkyl derivatives 78 and 79 were inactive as were sulfonamide 82, thiourea 84, and thioamide 83, suggesting the importance of acylation at this position. The sum of these results pointed to the desirable presence of moderately bulky cycloalkyl amide moieties in region II for good activity.

Evaluation Of The Benzopyran Region III SAR

[0049] Having thoroughly examined regions I and II, the modification of region III was then undertaken. Thus, compounds 85-102 (structures shown below, along with compound 68 for ease of comparison) were prepared (see Examples 8 and 9) and tested.



$R^1, R^2 =$



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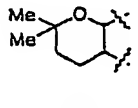

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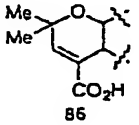

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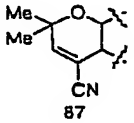

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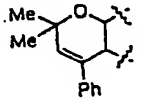

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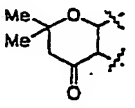

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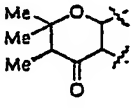

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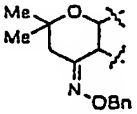

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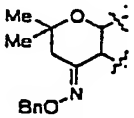

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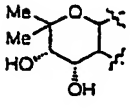

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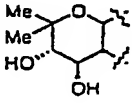

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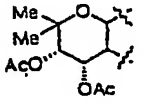

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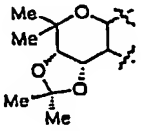

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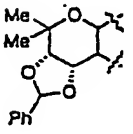

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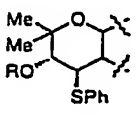
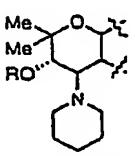


98: R = H
99: R = Ac



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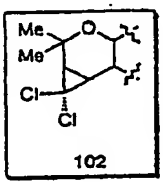
101: R = Ac



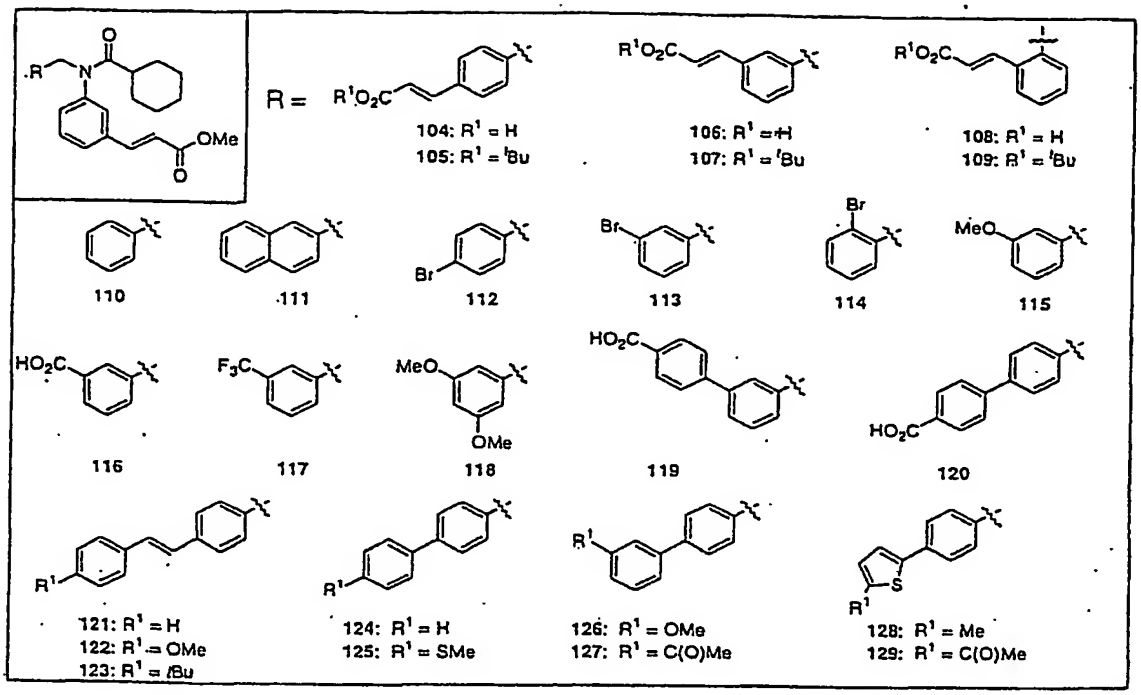
102

[0050] Incorporation of polar H-bond donating functional groups such as those that adorn compounds **86, 93, 94, 98** and **100** did not improve the activity of the analogs. Nor did the addition of H-bond acceptors such as in **89, 90, 95, 99** and **101** improve the ability of the parent compound **68** to activate FXR.

[0051] The addition of bulky lipophilic groups to the benzopyran moiety afforded compounds that only weakly activated FXR. However, replacement of the double bond in the benzopyran unit by a dichlorocyclopropane unit provided analog **102** (EC_{50} = 333 nM). Replacement of the benzoyl group in region II of compound **102** with the cyclohexylcarbonyl moiety afforded the even more potent compound **149** (EC_{50} = 188 nM).

[0052] Although compound **149** (EC_{50} = 188 nM) represents a significant improvement in potency over compound **65** (EC_{50} = 348 nM), it was not readily apparent how the activity of this class of compounds could be further improved. Therefore, it was decided to examine the effect of replacing the benzopyran moiety with other ring systems.

[0053] Thus, a series of compounds (i.e., Compounds **104-129**) in which the benzopyran moiety was replaced with certain groups of varying molecular diversity was prepared (see Examples 10 to 17) and tested.



[0054] Biological assays showed that replacement of the benzopyran with a small aromatic unit generally had a detrimental effect on activity. For instance, compounds **110** and **112 – 117** were inactive, while compounds **111** and **118** showed only moderate activation of FXR (EC_{50} = 680 nM and 606 nM, respectively; see Table 3 in Example 1). However, replacement of the benzopyran with an aromatic ring bearing substituents at the para position produced compounds with improved activity. For example, 4-tert-butyl cinnamate **105** (EC_{50} = 127 nM), stilbenes **121** and **122** (EC_{50} = 36 and 208 nM, respectively), biaryls **124 – 127** (EC_{50} = 510, 69, 77, 227 nM, respectively) and aryl thiophenes **128** and **129** (EC_{50} = 206 and 256 nM, respectively) were all potent activators of FXR in the cell-based reporter assay (see Tables 1, 9, 10 and 11 in Example 1).

[0055] This initial survey of the three regions of SAR outlined above led to the identification of several potent FXR agonists for further evaluation. One such agonist is the benzopyran-derived dichlorocyclopropane **149** (EC_{50} = 188 nM). Compound **105** (EC_{50} = 127 nM) is an example of a bis-cinnamate derivative. Finally, compounds **121** (EC_{50} = 36

nM) and **124** (EC_{50} = 69 nM) are stilbene and biaryl derivatives, respectively, of invention compounds.

[0056] Based on the results observed thus far, compound **149** appeared to represent the most potent derivative that could be readily obtained among the benzopyran-derivatives. However, the bis-cinnamate, biaryl, and stilbene derivatives of invention compounds were thought to still possess considerable potential for further development and rigorous SAR analysis. Below the results of such investigations are detailed, which indeed led to further enhancement of biological activity.

Examination Of The Bis-Cinnamate Series

[0057] Similar to the results described above, the meta substituted methyl cinnamate moiety on the "right-hand" region of the molecule remained a desirable component for elevated activity among the bis-cinnamate derivatives of invention compounds (see compounds **105** and **133-139**).

[0058] Replacement of the methyl acrylate unit with either a methyl or ethyl allylic ether (compounds **136** and **137**) caused only a slight decrease in activity (EC_{50} = 243 and 220 nM, respectively). A marked decline in potency accompanied substitution of the methyl acrylate by more sterically bulky ethers or esters (compounds **133** and **134**) or amides (compound **135**). Interestingly, saturation of the acrylate olefin (compound **139**) afforded only a two-fold decrease in potency, EC_{50} = 274 nM, which supports the notion that conformational rigidity is a factor contributing to, but not essential for, high affinity ligands. Importantly, compound **139** suggests that the methyl acrylate moiety is not simply functioning as a latent electrophile.

[0059] Region II also closely mirrored the preceding data as cycloalkyl amides remained the preferred substituents (see, compounds **105** and **140 – 145**: EC_{50} = 127 – 250 nM) among the bis-cinnamate derivatives of invention compounds. Aromatic and heterocyclic amides as well as alkyl ureas led to moderate potency (compounds **143 – 145**: EC_{50} = 205 – 236 nM) whereas incorporation of bulky ureas such as compound **146** rendered compounds of only marginal efficacy.

[0060] As mentioned above, replacement of the benzopyran moiety with a benzyl group bearing a tert-butyl acrylate moiety in the para-position yielded compound **105** with dramatically increased efficacy (EC_{50} = 127 nM). Interestingly, placement of the same tert-butyl acrylate group in either the meta or ortho positions of the aromatic ring in Region III led to only micromolar potency (see compounds **107** and **109**). Further investigation of the "left-hand" region in this series of compounds demonstrated that a decrease in ester group size yielded a corresponding decrease in efficacy (EC_{50} of t-butyl > i-propyl > ethyl > methyl (see, compounds **105** and **150 – 152**). Similarly, substitution of the ester with either carboxylic acid or amide functionality provided less effective compounds with EC_{50} values in the micromolar range. Substitution of the tert-butyl acrylate moiety with a methyl or ethyl allylic ether (see, compounds **156** and **157**) retained considerable potency (EC_{50} = 233 and 198 nM, respectively). However, the more bulky phenyl allylic ether **158** possessed only micromolar activity. In addition, saturation of the acrylate moiety (compound **159**) showed a two-fold decrease in potency from the parent compound **105**. Finally, substitution of the ortho position of the aromatic ring of the tert-butyl acrylate series with oxygenated functionality afforded compounds with very low biological activity (see compounds **161 – 167**).

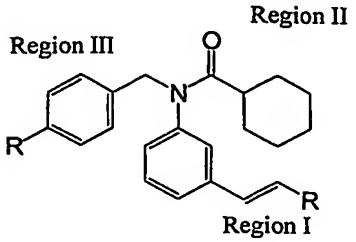
Construction of Biaryl and Stilbene Containing Focused Libraries

[0061] In an effort to further explore the activities of biaryl and stilbene derivatives of invention compounds, a 94-membered library of such compounds was constructed employing a solid phase strategy (see Example 18).

[0062] The selection of appropriate styrenes and boronic acids for inputs into this combinatorial library was guided by initial comparisons of tert-butyl stilbene (compound **123**, EC_{50} = > 1000 nM) to the unsubstituted stilbene **102** (EC_{50} = 36 nM), and biaryl compound **124** (EC_{50} = 510 nM) to compound **125** (EC_{50} = 69 nM). It was reasoned that both the stilbene and the biaryl ligands needed to fit into the same region of space within the receptor site for potent activation. Thus, stilbenes in which the aromatic nucleus is removed two carbon atoms further away from the core of the molecule should be adorned with small substituents while the biaryl compounds should be adorned with larger functionality for optimal activity. Screening of this compound library in the cell-based assay led to some intriguing results as summarized in Tables 12-15 of Example 1.

[0063] Thus, it was found that in both stilbene and biaryl derivatives of invention compounds, analogs bearing the cyclohexylamide moiety are generally the most potent followed by those bearing the isopropyl amide or isopropyl urea units. As predicted above, stilbenes bearing smaller substituents were more potent than those bearing larger functionality. For instance, substituted stilbene **121** and mono-fluoro stilbenes **192**, **201**, and **204** were among the most active, while mono-methyl derivative **174** and tri-methyl derivative **195** were among the least active. Also of interest were heterocyclic compounds **207** and **210**, which retained good potency (EC_{50} = 309 and 227 nM, respectively) and may possess improved pharmacological properties. With biaryl derivatives of invention compounds, compounds which present more bulk at the terminus of the structure were more active. With these derivatives, compounds **259** (EC_{50} = 25 nM) and **244** (EC_{50} = 38 nM) were particularly active. Overall, most of the compounds synthesized in this follow-up library were efficient activators of FXR, confirming the accuracy of the working hypothesis for the FXR binding pocket described above, which provides a solid basis for further development of FXR activators.

[0064] A summary of the molecular requirements for potent FXR activation is shown below:



Thus, in Region I the presence of the meta methyl acrylate unit or allylic methyl ether is desirable for potent activation as only a few modifications retain good activity. In some instances, when other areas are modified, the olefin can be deleted from Region I while retaining potency. The most potent compounds observed thus far possess an amide or a urea in Region II. Presently most preferred compounds have a cycloalkylamide or a cycloalkylurea in Region II. Finally, Region III is the most tolerant, indeed, several structural elements were found to provide a good fit within the pocket of the receptor. Generally, it is desirable for the aromatic ring to be para-substituted, with the steric bulk and length of the substituent imparting a significant effect on potency of the resulting compound.

[0065] In order to determine how selectively the above-described compounds activated FXR, some of the most active compounds were screened against a panel of nuclear receptors. Most of these compounds were found to be selective for activation only of FXR. Notably, however, compound **149** also potently activated SXR. This result may lead to compounds which have utility in the treatment of diseases linked to the accumulation of toxic bile acids.

[0066] In accordance with another embodiment of the present invention, there are provided formulations comprising at least one of the above-described compounds in a pharmaceutically acceptable carrier therefor. Exemplary pharmaceutically acceptable carriers include solids, solutions, emulsions, dispersions, micelles, liposomes, and the like. Optionally, the pharmaceutically acceptable carrier employed herein further comprises an enteric coating.

[0067] Pharmaceutically acceptable carriers contemplated for use in the practice of the present invention are those which render invention compounds amenable to oral delivery, transdermal delivery, intravenous delivery, intramuscular delivery, topical delivery, nasal delivery, and the like.

[0068] Thus, formulations of the present invention can be used in the form of a solid, a solution, an emulsion, a dispersion, a micelle, a liposome, and the like, wherein the resulting formulation contains one or more of the compounds of the present invention, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for enterable or parenteral applications. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, solutions, emulsions, suspensions and any other suitable for use. The carriers which can be used include glucose, lactose, gum acacia, gelatin, manitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, medium chain length triglycerides, dextrans, and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form. In addition auxiliary, stabilizing, thickening, and coloring agents and perfumes may be used. The active compound(s) is (are) included in the formulation in an amount sufficient to produce the desired effect upon the process or disease condition.

[0069] Invention formulations containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Formulations intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such formulations may contain one or more agents selected from the group consisting of a sweetening agent such as sucrose, lactose, or saccharin, flavoring agents such as peppermint, oil of wintergreen or cherry, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients used may be, for example (1) inert diluents such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents such corn starch, potato starch or alginic acid; (3) binding agents such

as gum tragacanth, corn starch, gelatin or acacia, and (4) lubricating agents such as magnesium stearate, steric acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by such techniques as those described in U.S. Pat Nos. 4,256,108; 4,160,452; and 4,265,874, to form osmotic therapeutic tablets for controlled release.

[0070] In some cases, formulations contemplated for oral use may be in the form of hard gelatin capsules wherein the active ingredient is mixed with inert solid diluent(s), for example, calcium carbonate, calcium phosphate or kaolin. They may also be in the form of soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil.

[0071] Invention formulations may be in the form of a sterile injectable suspension. This suspension may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides, fatty acids, naturally occurring vegetable oils like sesame oil, coconut oil, peanut oil, cottonseed oil, etc. or synthetic fatty vehicles like ethyl oleate or the like. Buffers, preservatives, antioxidants, and the like can be incorporated as required.

[0072] Invention formulations may also be administered in the form of suppositories for rectal administration of the drug. These formulations may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters of polyethylene glycols, which are solid at ordinary temperatures, but liquefy and/or dissolve in the rectal cavity to release the drug. Since individual subjects may present a wide variation in severity of symptoms and each drug has its unique therapeutic characteristics, the precise

mode of administration and dosage employed for each subject is left to the discretion of the practitioner.

[0073] Amounts effective for the particular therapeutic goal sought will, of course, depend on the severity of the condition being treated, and the weight and general state of the subject. Various general considerations taken into account in determining the "effective amount" are known to those of skill in the art and are described, e.g., in Gilman *et al.*, eds., Goodman And Gilman's: The Pharmacological Bases of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990, each of which is herein incorporated by reference.

[0074] The term "effective amount" as applied to invention compounds, means the quantity necessary to effect the desired therapeutic result, for example, a level effective to treat, cure, or alleviate the symptoms of a disease state for which the therapeutic compound is being administered, or to establish homeostasis. Since individual subjects may present a wide variation in severity of symptoms and each drug or active agent has its unique therapeutic characteristics, the precise mode of administration, dosage employed and treatment protocol for each subject is left to the discretion of the practitioner.

[0075] In accordance with yet another embodiment of the present invention, there are provided methods for modulating process(es) mediated by farnesoid X receptor polypeptides, said methods comprising conducting said process(es) in the presence of an effective amount of at least one compound according to the invention.

[0076] As employed herein, "modulating" refers to the ability of a modulator for a member of the nuclear receptor superfamily (*e.g.*, FXR) to either directly (by binding to the receptor as a ligand) or indirectly (as a precursor for a ligand or an inducer which promotes production of ligand from a precursor) induce expression of gene(s) maintained under hormone expression control, or to repress expression of gene(s) maintained under such control. Exemplary processes contemplated for modulation according to the invention include cholesterol metabolism, regulation of lipid homeostasis, stimulation of bile transport and absorption, regulation of the expression of genes involved in the excretion and

transportation of bile acids (including intestinal bile acid-binding protein (IBABP)), bile salt export pump (BSEP) and canalicular multi-specific organic anion transporter (cMOAT), and the like.

[0077] Bile acids are derivatives of cholesterol synthesized in the hepatocyte. Cholesterol, ingested as part of the diet or derived from hepatic synthesis is converted into the bile acids cholic and chenodeoxycholic acids, which are then conjugated to an amino acid (glycine or taurine) to yield the conjugated form that is actively secreted into cannaliculi. Bile acids are facial amphipathic, that is, they contain both hydrophobic (lipid soluble) and polar (hydrophilic) faces. The cholesterol-derived portion of a bile acid has one face that is hydrophobic (that with methyl groups) and one that is hydrophilic (that with the hydroxyl groups); the amino acid conjugate is polar and hydrophilic. Therefore, compounds that can be used to modulate such pathways via effects on FXR involving bile acids are useful in cholesterol metabolism.

[0078] Bile acid synthesis is a major pathway for cholesterol disposal and thus represents a potential therapeutic target pathway for the treatment of hypercholesterolemia. FXR acts as a bile acid receptor and biological sensor for the regulation of bile acid biosynthesis. FXR is known to regulate cholesterol metabolism in two ways: (1) chenodeoxycholic acid (CDCA), a primary bile acid, binds directly to and activates FXR, which then mediates the feedback suppression by bile acids of cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid biosynthesis from cholesterol; and (2) FXR participates in the activation of intestinal bile acid binding protein (IBABP), which is involved in the enterohepatic circulation of bile acids. Thus FXR constitutes a potential therapeutic target that can be modulated to enhance the removal of cholesterol from the body. Novel compounds identified by the methods presented herein provide a new tool for regulating or modulating FXR function.

[0079] Furthermore, FXR is known to in turn activate a series of target genes. In particular FXR functions as a heterodimer with the 9-cis-retinoic acid receptor (RXR). A number of target DNA binding sequences that would be present in target genes have recently been identified. A consensus sequence has been determined, which contains an inverted

repeat of the sequence AGGTCA with a 1-base pair spacing (IR-1) (Laffitte *et al.*, *J. Biol. Chem.* **275**:10638-10647 (2000). This sequence was shown to be a high affinity binding site for FXR/RXR *in vitro* and to confer ligand-dependent transcriptional activation by FXR/RXR to a heterologous promoter in response to a bile acid or synthetic retinoid. Although these studies demonstrated that the FXR/RXR heterodimer binds to the consensus IR-1 sequence with the highest affinity, it was also demonstrated that FXR/RXR can bind to and activate through a variety of elements including IR-1 elements with changes in the core half-site sequence, spacing nucleotide, and flanking nucleotides. In addition, it was shown that FXR/RXR can bind to and transactivate through direct repeats. Therefore, by providing novel ways to modulate FXR function, the present invention in turn provides a method of modulating the function of a variety of target genes that are acted upon by FXR.

[0080] In accordance with still another embodiment of the present invention, there are provided methods for the treatment of hypercholestemia, said methods comprising administering an effective amount of at least one compound according to the invention to a subject in need thereof.

[0081] In accordance with still another embodiment of the present invention, there are provided methods for the treatment of cholestasis, said methods comprising administering an effective amount of at least one compound according to the invention to a subject in need thereof.

[0082] The invention will now be described in greater detail with reference to the following non-limiting Examples.

EXAMPLE 1

In vivo assay

[0083] The feasibility of creating high throughput screens (HTS) for ORs was explored using FXR as a candidate orphan receptor (OR) with a known activator, chenodeoxycholic acid (CDCA) as a ligand. The screen is based on the co-transfection of a full-length receptor with the reporter vector containing a natural hormone response element under a minimal eukaryotic promoter. The results provided herein (see, Figure 1) demonstrate that

compounds can be successfully screened in a dose dependent manner for potential activating chemical ligands using a full length FXR on a natural response element. These results validate the robustness of the assay for FXR, in 384-well plates. Using this 384-well format, the high throughput screening (HTS) approach to FXR as a candidate OR was employed. For this test screen, a 10,000 membered library, constructed around the privileged 2,2-dimethylbenzopyran scaffold, was employed (see Nicolaou *et al., J. Am. Chem. Soc.* **122**:9939-9953 and 9954-9967 (2000)). This library comprises approximately 10,000 distinct compounds with structures and sizes similar to natural products such as phyto-estrogens, flavanoids, coumarins and long chain fatty acids. A central question in the feasibility studies is whether this library is suitable for screening for nuclear receptor ligands. Samples of this library were first reformatted into a 384-well format and then subjected to the FXR cell-based assays described above and assessed for FXR-mediated transcriptional activity. Cells were exposed to approximately 10 μM of sample for 18 hrs prior to washing and luciferase analysis.

[0084] The 25 most active compounds at 10 μM were re-synthesized to confirm their structure and activity. Smaller "focused" chemical libraries were then designed and prepared around these hits and subjected to multiple rounds of screening. Through this iterative process a total of seven additional rounds of synthesis and selection was conducted resulting in novel compounds that are as effective as a proprietary synthetic ligand developed by Glaxo-Smith-Kline (GW 4064) in cell based assays. Using one of these identified compounds, fexaramate (**105**; EC_{50} 127 nM), as a scaffold, three additional focused libraries were made and screened to obtain at least four potent, non-steroidal FXR agonists termed fexarene (**121**; EC_{50}, 36 nM), fexaramine (**259**; EC_{50}, 25 nM), fexarine (**244**; EC_{50}, 38 nM) and fexarchloramide (**149**; EC_{50}, 188 nM). EC_{50} values were determined with Prism 3.0 software via the activity of the subject compound in the previously described cell based assay.

[0085] EC_{50} values for the "scaffold" compound, fexaramate (**105**), and numerous variations thereof, are presented below (see Tables 1-11).

a. SAR of region I

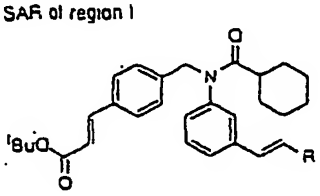
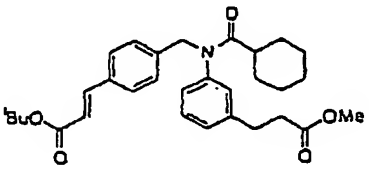


Table 1

	R	EC_{50} (nM)	RE[a]
105	COOMe	127	2.12
133	COOEt	256	2.07
134	COO^tBu	>1000	1.06
135	$CONH_2$	>1000	0.50
136	CH_2OMe	243	1.88
137	CH_2OEt	220	1.74
138	CH_2OPh	2830	0.45



139: EC_{50} = 274 nM
RE[a] = 1.38

b. SAR of region II

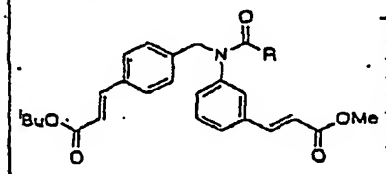


Table 2

	R	EC_{50} (nM)	RE[a]
140	cyclopropyl	250	1.68
141	cyclobutyl	187	1.84
142	cyclopentyl	162	2.16
105	cyclohexyl	127	2.12
143	phenyl	238	1.96
144	2-furyl	205	1.93
145	isopropylamino	212	1.96
146	benzylamino	>1000	0.27

c. SAR of region III

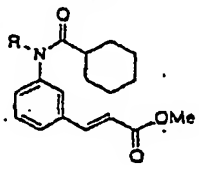


Table 3

	R	EC_{50} (nM)	RE[a]
132	H	>1000	0.09
147	methyl	>1000	0.09
110	benzyl	>1000	0.09
111	2-napthyl	680	0.41
114	2-bromobenzyl	>1000	0.11
113	3-bromobenzyl	>1000	0.10
112	4-bromobenzyl	>1000	0.28
148	4-*tert*-butylbenzyl	>1000	0.15
115	3-methoxybenzyl	>1000	0.11
118	3,5-dimethoxybenzyl	606	0.11
117	3-(trifluoromethyl)benzyl	>1000	0.12

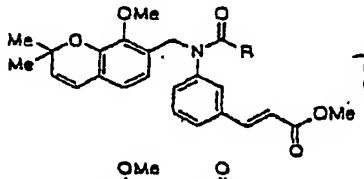


Table 4

	R	EC_{50} (nM)	RE[a]
68	phenyl	>1000	0.83
65	cyclohexyl	358	0.40

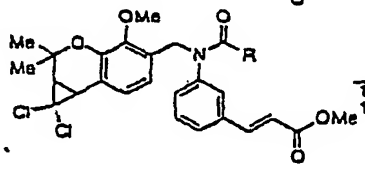


Table 5

	R	EC_{50} (nM)	RE[a]
102	phenyl	333	0.64
149	cyclohexyl	188	0.50

d. SAR of region III

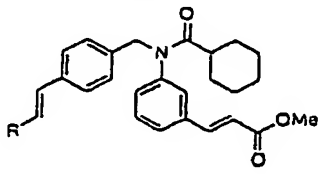


Table 6

	R	EC_{50} (nM)	RE[a]
104	COOH	>1000	0.08
150	COOMe	>1000	0.87
151	COOEt	>1000	1.14
152	COOPr	163	1.97
105	COO^{t}Bu	127	2.12
153	COOBn	>1000	0.23
154	$CONMe_2$	>1000	0.66
155	CONH^{t}Bu	>1000	1.65
156	CH_2OMe	233	1.63
157	CH_2OEt	198	2.06
158	CH_2OPh	>1000	0.64

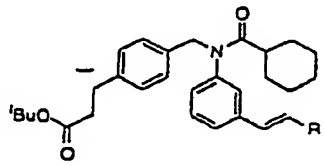
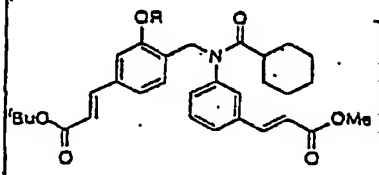


Table 7

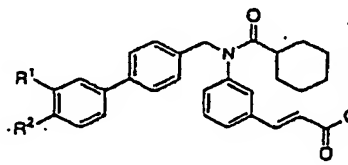
	R	EC_{50} (nM)	RE[a]
159	COOMe	240	1.56
160	COO^{t}Bu	>1000	0.64

Table 8



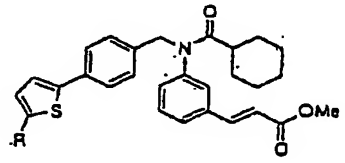
	R	EC_{50} (nM)	RE[a]
161	H	> 1000	0.12
162	Me	> 1000	0.14
163	Bn	> 1000	0.38
164	MeC(O)	> 1000	0.16
165	$C_6H_5C(O)$	> 1000	0.18
166	$MeS(O_2)$	> 1000	0.18
167	$EtOOCCH_2$	> 1000	0.18

Table 9



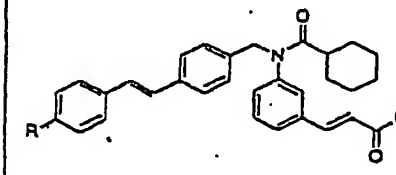
	R^1	R^2	EC_{50} (nM)	RE[a]
1	OMe	H	77	1.51
127	C(O)Me	H	227	1.30
125	H	SMe	69	1.74
124	H	H	510	0.71

Table 10



	R	EC_{50} (nM)	RE[a]
128	Me	206	1.78
129	C(O)Me	256	1.48

Table 11



	R	EC_{50} (nM)	RE[a]
121	H	36	1.55
122	OMe	208	1.67
123	t-Bu	>1000	0.29

[0086] Several of the derivatives set forth above are seen to possess excellent activity (e.g., Compounds **121, 125, 141, 142,** etc.).

[0087] EC_{50} values for numerous additional variations of the compounds presented above are presented below (see Tables 12-15).

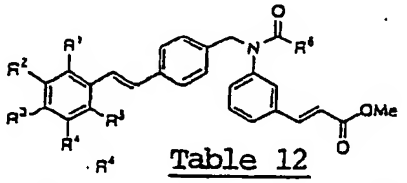


Table 12

	R^1	R^2	R^3	R^4	R^5	R^6	EC_{50} (nM)	RE[a]
174	H	H	Me	H	H	$-C_6H_{11}$	342	0.83
175	H	H	Me	H	H	$-CH(CH_3)_2$	1410	0.37
176	H	H	Me	H	H	$-NHCH(CH_3)_2$	3570	0.10
177	Cl	H	H	H	Cl	$-C_6H_{11}$	150	0.12
178	Cl	H	H	H	Cl	$-CH(CH_3)_2$	195	0.14
179	Cl	H	H	H	Cl	$-NHCH(CH_3)_2$	216	0.15
180	H	Cl	H	H	H	$-C_6H_{11}$	165	1.41
181	H	Cl	H	H	H	$-CH(CH_3)_2$	164	1.09
182	H	Cl	H	H	H	$-NHCH(CH_3)_2$	339	0.59
183	H	CF_3	H	CF_3	H	$-C_6H_{11}$	1470	0.15
184	H	CF_3	H	CF_3	H	$-CH(CH_3)_2$	1950	0.13
185	H	CF_3	H	CF_3	H	$-NHCH(CH_3)_2$	1830	0.13
186	H	CF_3	H	H	H	$-C_6H_{11}$	937	0.35
187	H	CF_3	H	H	H	$-CH(CH_3)_2$	267	0.70
188	H	CF_3	H	H	H	$-NHCH(CH_3)_2$	932	0.31
189	F	H	H	H	F	$-C_6H_{11}$	174	0.94
190	F	H	H	H	F	$-CH(CH_3)_2$	108	0.79
191	F	H	H	H	F	$-NHCH(CH_3)_2$	4020	0.21
192	F	H	H	H	H	$-C_6H_{11}$	64	1.41
193	F	H	H	H	H	$-CH(CH_3)_2$	70	1.17
194	F	H	H	H	H	$-NHCH(CH_3)_2$	431	0.69
195	Me	H	Me	H	Me	$-C_6H_{11}$	518	0.24
196	Me	H	Me	H	Me	$-CH(CH_3)_2$	149	0.30
197	Me	H	Me	H	Me	$-NHCH(CH_3)_2$	431	0.14
121	H	H	H	H	H	$-C_6H_{11}$	36	1.55
198	H	H	H	H	H	$-CH(CH_3)_2$	65	1.33
200	H	H	H	H	H	$-NHCH(CH_3)_2$	119	1.38
201	H	F	H	H	H	$-C_6H_{11}$	86	1.38
202	H	F	H	H	H	$-CH(CH_3)_2$	71	1.33
203	H	F	H	H	H	$-NHCH(CH_3)_2$	467	0.61
204	H	H	F	H	H	$-C_6H_{11}$	185	0.53
205	H	H	F	H	H	$-CH(CH_3)_2$	120	1.19
206	H	H	F	H	H	$-NHCH(CH_3)_2$	348	0.91

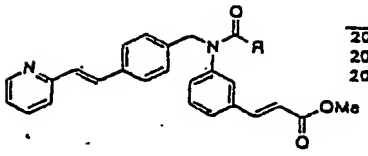
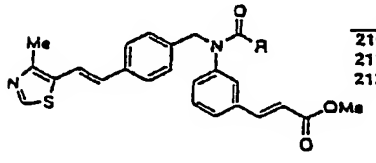


Table 13

	R	EC_{50} (nM)	RE[a]
207	$-C_6H_{11}$	309	0.81
208	$-CH(CH_3)_2$	310	0.62
209	$-NHCH(CH_3)_2$	575	0.68

Table 14



	R	EC_{50} (nM)	RE[a]
210	$-C_6H_{11}$	227	0.53
211	$-CH(CH_3)_2$	228	0.32
212	$-NHCH(CH_3)_2$	366	0.42

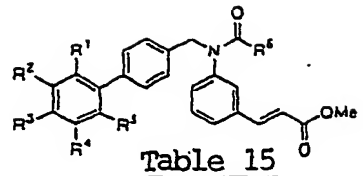


Table 15

	R¹	R²	R³	R⁴	R⁵	R⁶	EC₅₀ (nM)	RE⁵
213	H	F	F	H	H	-C_6H_{11}	72	1.70
214	H	F	F	H	H	-$CH(CH_3)_2$	249	1.15
215	H	F	F	H	H	-$NHCH(CH_3)_2$	8180	0.23
125	H	H	SMe	H	H	-C_6H_{11}	69	1.74
216	H	H	SMe	H	H	-$CH(CH_3)_2$	51	0.98
217	H	H	SMe	H	H	-$NHCH(CH_3)_2$	178	0.23
218	OMe	H	H	H	H	-C_6H_{11}	359	0.49
219	OMe	H	H	H	H	-$CH(CH_3)_2$	377	0.28
220	OMe	H	H	H	H	-$NHCH(CH_3)_2$	4010	0.09
126	H	Cl	H	Cl	H	-C_6H_{11}	284	0.95
221	H	Cl	H	Cl	H	-$CH(CH_3)_2$	661	0.54
222	H	Cl	H	Cl	H	-$NHCH(CH_3)_2$	>10000	0.10
223	H	OMe	H	H	H	-C_6H_{11}	101	1.51
224	H	OMe	H	H	H	-$CH(CH_3)_2$	72	1.26
225	H	OMe	H	H	H	-$NHCH(CH_3)_2$	1370	0.41
226	H	OEt	H	H	H	-C_6H_{11}	147	1.37
227	H	OEt	H	H	H	-$CH(CH_3)_2$	173	1.03
228	H	OEt	H	H	H	-$NHCH(CH_3)_2$	2350	0.33
229	H	H	OMe	H	H	-C_6H_{11}	89	1.71
230	H	H	OMe	H	H	-$CH(CH_3)_2$	97	1.21
231	H	H	OMe	H	H	-$NHCH(CH_3)_2$	144	1.16
232	H	Cl	H	H	H	-C_6H_{11}	94	1.56
233	H	Cl	H	H	H	-$CH(CH_3)_2$	77	1.52
234	H	Cl	H	H	H	-$NHCH(CH_3)_2$	1400	0.49
235	H	H	Me	H	H	-C_6H_{11}	26	1.38
236	H	H	Me	H	H	-$CH(CH_3)_2$	118	1.48
237	H	H	Me	H	H	-$NHCH(CH_3)_2$	449	0.80
238	H	Me	H	H	H	-C_6H_{11}	109	1.43
239	H	Me	H	H	H	-$CH(CH_3)_2$	163	1.09
240	H	Me	H	H	H	-$NHCH(CH_3)_2$	1330	0.53
241	OMe	H	H	Cl	H	-C_6H_{11}	233	1.16
242	OMe	H	H	Cl	H	-$CH(CH_3)_2$	226	0.79
243	OMe	H	H	Cl	H	-$NHCH(CH_3)_2$	3080	0.17
244	H	-OCH_2O-		H	H	-C_6H_{11}	38	1.90
245	H	-OCH_2O-		H	H	-$CH(CH_3)_2$	18	1.25
246	H	-OCH_2O-		H	H	-$NHCH(CH_3)_2$	96	1.51
247	H	Cl	F	H	H	-C_6H_{11}	66	1.87
248	H	Cl	F	H	H	-$CH(CH_3)_2$	129	1.64
249	H	Cl	F	H	H	-$NHCH(CH_3)_2$	3050	0.41
250	H	H	OCF_3	H	H	-C_6H_{11}	264	1.04
251	H	H	OCF_3	H	H	-$CH(CH_3)_2$	219	0.78
252	H	H	OCF_3	H	H	-$NHCH(CH_3)_2$	7530	0.21
253	H	OCF_3	H	H	H	-C_6H_{11}	420	0.84
254	H	OCF_3	H	H	H	-$CH(CH_3)_2$	247	0.59
255	H	OCF_3	H	H	H	-$NHCH(CH_3)_2$	>10000	0.09
256	OMe	H	H	H	OMe	-C_6H_{11}	77	0.12
257	OMe	H	H	H	OMe	-$CH(CH_3)_2$	95	0.10
258	OMe	H	H	H	OMe	-$NHCH(CH_3)_2$	561	0.10
259	H	H	NMe_2	H	H	-C_6H_{11}	25	1.72
260	H	H	NMe_2	H	H	-$CH(CH_3)_2$	57	1.07
261	H	H	NMe_2	H	H	-$NHCH(CH_3)_2$	162	1.01
262	H	H	t-Bu	H	H	-C_6H_{11}	132	1.38
263	H	H	t-Bu	H	H	-$CH(CH_3)_2$	340	0.59
264	H	H	t-Bu	H	H	-$NHCH(CH_3)_2$	282	1.02

[0088] Several of the derivatives set forth above are seen to possess excellent activity (e.g., Compounds 177, 180, 181, 189, 190, 192, 193, 198, 201, 202, 204, 205, 213, 216, 224, 229, 230, 232, 233, 235, 236, 238, 244, 245, 246, 247, 256, 257, 259, 260, etc.).

EXAMPLE 2

In vitro Screening

[0089] An *in vitro* based "proximity" screen is an excellent complement to live cell assays and can be used as a measure of direct ligand binding. Hence this type of screen is also an effective measure of the affinity of binding without the use of a radiolabel. The approach employed herein is termed AlphaScreen technology. For this assay purified receptor protein is expressed as a glutathione S-transferase (GST) fusion protein and is bound via a GST antibody to a "donor" bead. This is then mixed with a biotinylated co-activator peptide that has been linked to an Avidin proximity sensitive "acceptor" bead. These reactants are mixed in a 384-well plate and are then exposed to either a known inducer (control) or an ordered array of unknown compounds (library). If the acceptor bead (linked to the co-activator peptide) is brought into close proximity of the donor bead, by virtue of a biological interaction, singlet-state oxygen molecules are released and react with chemiluminescent groups in the acceptor beads. The effect of either known inducers or candidate chemical compounds on the interaction of a receptor and its co-activator peptide can be measured by a change in the Alpha signal.

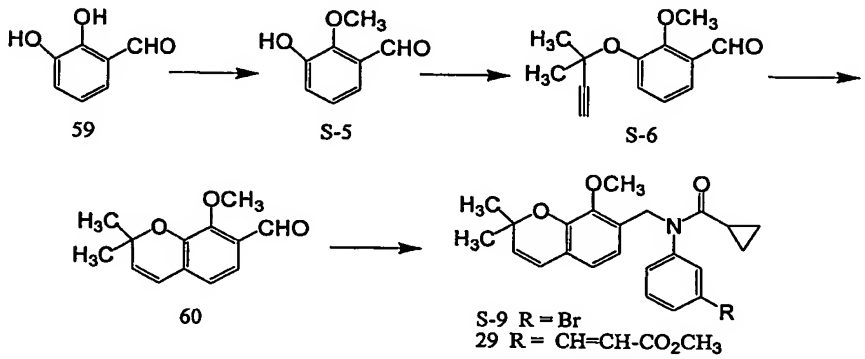
[0090] The ability of the *in vitro* AlphaQuest assay to detect receptor/co-activator peptide interactions in a 384 well format has been evaluated using the thyroid hormone receptor (TR) and the retinoid X receptor (RXR) as positive controls. The results demonstrate that receptor/co-activator peptide interactions can be detected in a dose-dependent manner with binding efficiencies similar to those reported in the literature, validating this as a critical *in vitro* approach to demonstrate binding of candidate ligands in the absence of a high affinity radiolabeled competitor.

[0091] Representative procedures for the preparation of Region I modified compounds are shown in Examples 3 to 6.

Example 3

Preparation of Compounds 29, 60, S-5, S-6 and S-9

[0092] The strategy employed for the preparation of Compound **29** is shown below.

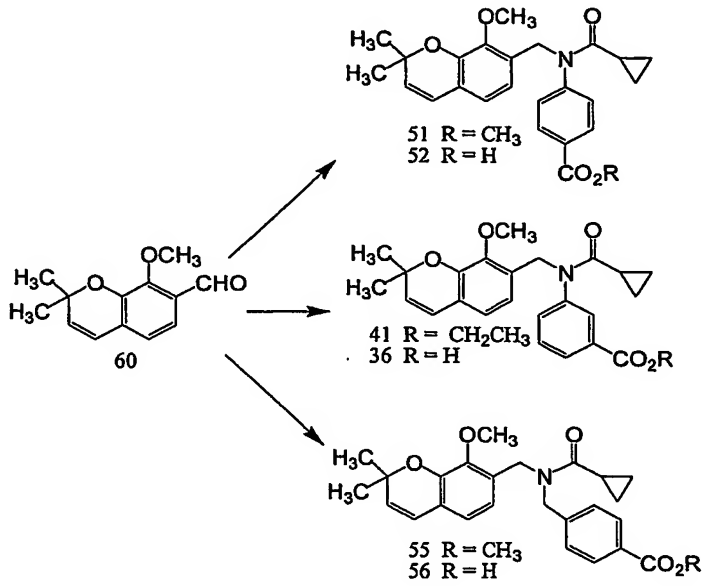


[0093] Thus, 2,3-dihydroxy benzaldehyde **59** is selectively methylated (NaH, MeI) to afford the monoalcohol benzaldehyde **S-5**. **S-5** is O-alkylated (1.5 equiv. of 2-methyl-3-butyn-2-ol, 1.7 equiv. of TFAA, 1.5 equiv. of DBU, 0.1 equiv. of $CuCl_2$, CH_3CN, 0-25°C, 12 h., 75%) to provide the propargyl ether **S-6**. **S-6** is thermally cyclized (N,N-diethylaniline, 190°C, 0.5 h., 90%) to afford the intermediate benzopyran **60**. **60** is reductively aminated (1.5 equiv. of 3-bromoaniline, THF, 70°C, 4 h., 90%, then 2.0 equiv. of $NaCNBH_3$, 10% MeOH, 70°C, 4 h., 83%) and the intermediate amine is acylated (1.3 equiv. of cyclopropanecarbonyl chloride (C_3H_5COCl), 1.3 equiv. of Et_3N, 0.1 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 12 h., 85-95%) to provide the aryl bromide amide **S-9**. **S-9** is coupled to methyl acrylate by a palladium-mediated Heck reaction (4.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.5 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 24 h., 80%) to afford compound **29**.

Example 4

Preparation of Compounds 28, 29, 36, 42, 51-56, S-7 and S-8

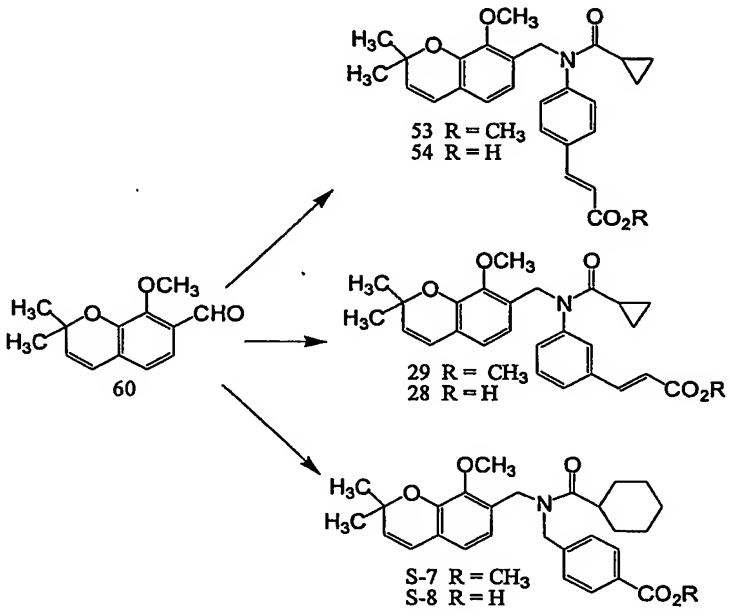
[0094] The strategy employed for the preparation of Compounds **28, 29, 36, 42, 51-56, S-7** and **S-8** is shown below.



[0095] Thus, benzopyran aldehyde **60** is reductively aminated (1.5 equiv. of methyl 4-amino-benzoate, or ethyl 3-aminobenzoate, or methyl (4-aminomethyl)benzoate, THF, 70°C, 4 h., 70°C, then 2.0 equiv. of $NaCNBH_3$, 10% MeOH, 70°C, 4 h., 77%-82%) and the intermediate amine is acylated (1.3 equiv. of cyclopropanecarbonyl chloride (C_3H_5COCl), 1.3 equiv. of Et_3N, 0.1 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 12 h., 85-95%) to afford amides **51, 41** and **55**, respectively. **51, 41** and **55** are subsequently hydrolyzed (4.0 equiv. of LiOH, THF:H_2O (10:1), 25°C, 12 h., 75%-98%) to provide the mono-acids **52, 36** and **56**, respectively.

[0096] Similarly, the reductive amination product of benzopyran aldehyde **60**, is acylated (1.3 equiv. of cyclopropanecarbonyl chloride (C_3H_5COCl), 1.3 equiv. of Et_3N, 0.1 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 12 h., 85-95%) to provide amides **53, 29** and **S-7**, respectively. **53,**

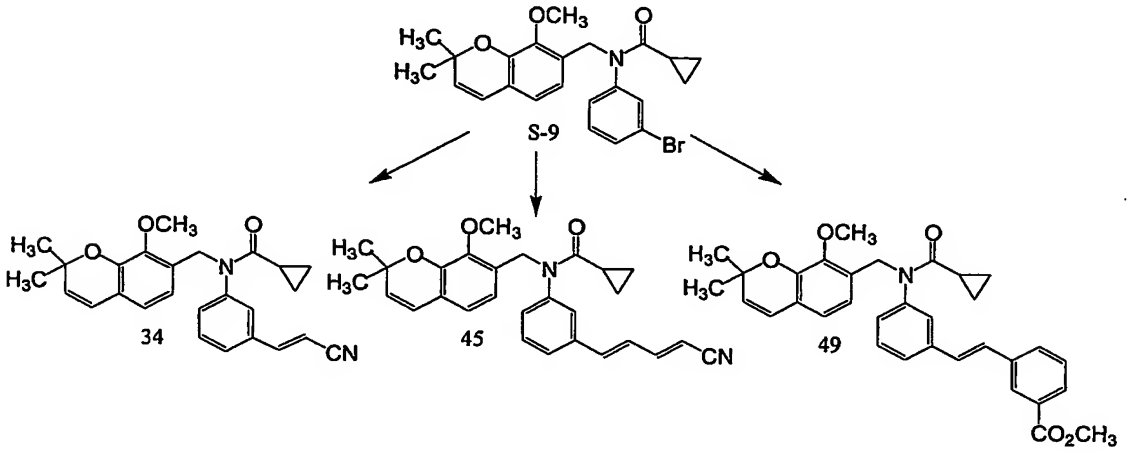
29 and **S-7** are hydrolyzed (4.0 equiv. of LiOH, THF:H_2O (10:1), 25°C, 12 h., 75%-98%) to afford the mono-acids, **54**, **28** and **S-8**, respectively.



Example 5

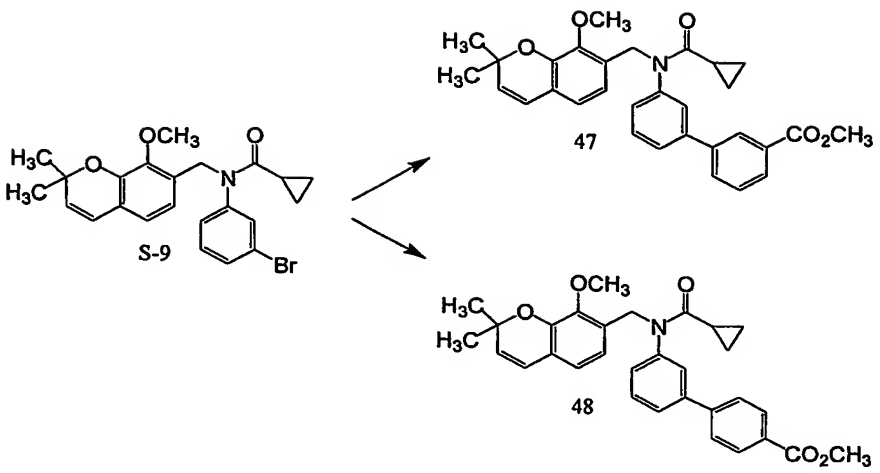
Preparation of Compounds 34, 45 and 47-49

[0097] The strategy employed for the preparation of Compounds **34**, **45** and **47-49** is shown below.



[0098] Thus, **S-9** is coupled by a palladium-mediated Heck reaction (2.0 equiv. of acrylonitrile or 2.0 equiv. of penta-2,4-dienoic acid methyl ester, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 55% and 70% and 85%) to afford compounds **34** and **45**, respectively.

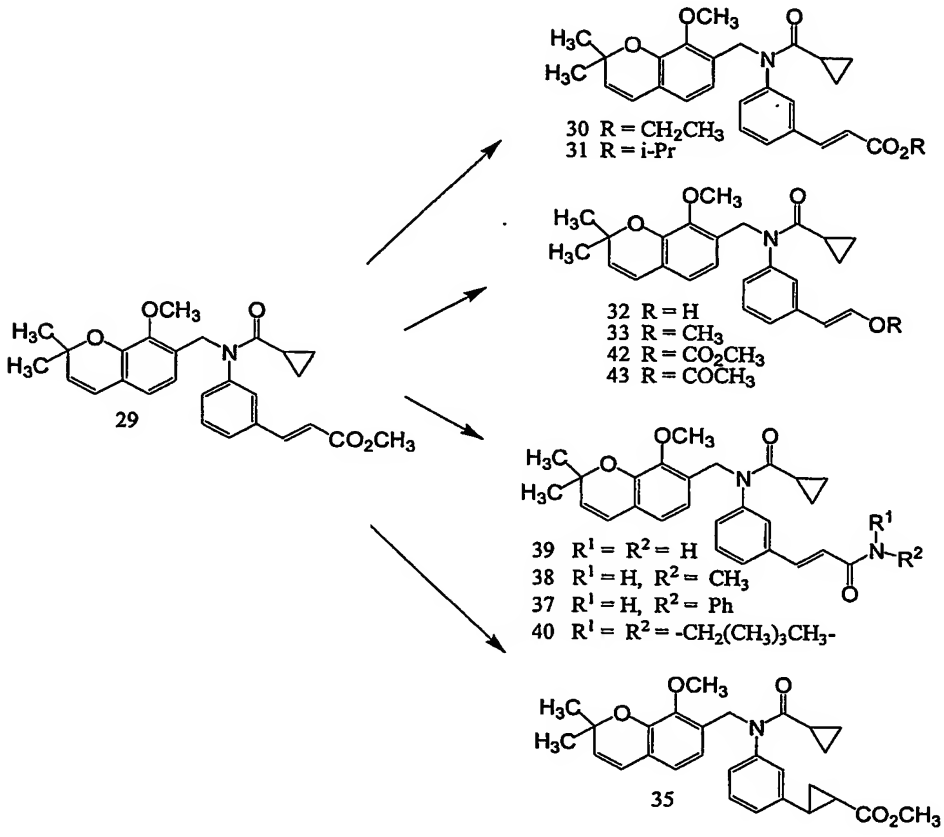
[0099] Similarly, **S-9** is coupled by a palladium-mediated Heck reaction (2.0 equiv. of 3-vinylbenzaldehyde, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 85%) to provide the coupled benzaldehyde, which is oxidized (1.5 equiv. of $NaClO_2$, 4.0 equiv. of NaH_2PO_4, 10 equiv. of 2-methyl-2-butene, THF:*t*-BuOH:H_2O (3:1:1), 25°C, 3 h., 98%) and the resulting acid is methylated (10.0 equiv. of CH_2N_2, Et_2O, 0°C, 1 h., 100%) to provide the methyl ester **48**. **S-9** also undergoes a palladium-mediated Suzuki reaction (5.0 equiv. of (3-methoxycarbonylphenyl)boronic acid or (4-methoxycarbonyl-phenyl) boronic acid, 0.2 equiv. of $Pd(PPh_3)_4$, toluene:MeOH:1M Na_2CO_3 (10:3:1), 90°C, 24 h., 75% and 78%) to afford biphenyls **47** and **48**, respectively.


OCH3
O
H3C
H3C
O
N
CO2CH3
47
S-9
Br
48

Example 6

Preparation of Compounds 30-33, 35, 37-40, 42 and 43

[0100] The strategy for the preparation of Compounds **30-33, 35, 37-40, 42** and **43** is shown below.



[0101] Thus, **29** is transesterified (0.5 equiv. of n-Bu_2Sn=O, EtOH, or *i*-PrOH, 25°C, 48 h., 50% and 34%) to afford esters **30** and **31**, respectively. **29** is reduced (1.2 equiv. of diisobutylaluminum hydride (Dibal-H), toluene, -78°C, 0.5 h., 52%) to provide the allyl alcohol **32**. **32** is O-alkylated (2.0 equiv. of NaH, 3.0 equiv. of MeI, 0°C, 1 h., 95%), or is acylated (1.2 equiv. of MeOC(O)Cl, 2.0 equiv. of Et_3N, 0.1 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 24 h., 90% or 1.2 equiv. of. MeC(O)Cl, 2.0 equiv. of Et_3N, 0.1 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 24 h., 90%) to afford compounds **33, 42** and **43**, respectively. **29** is

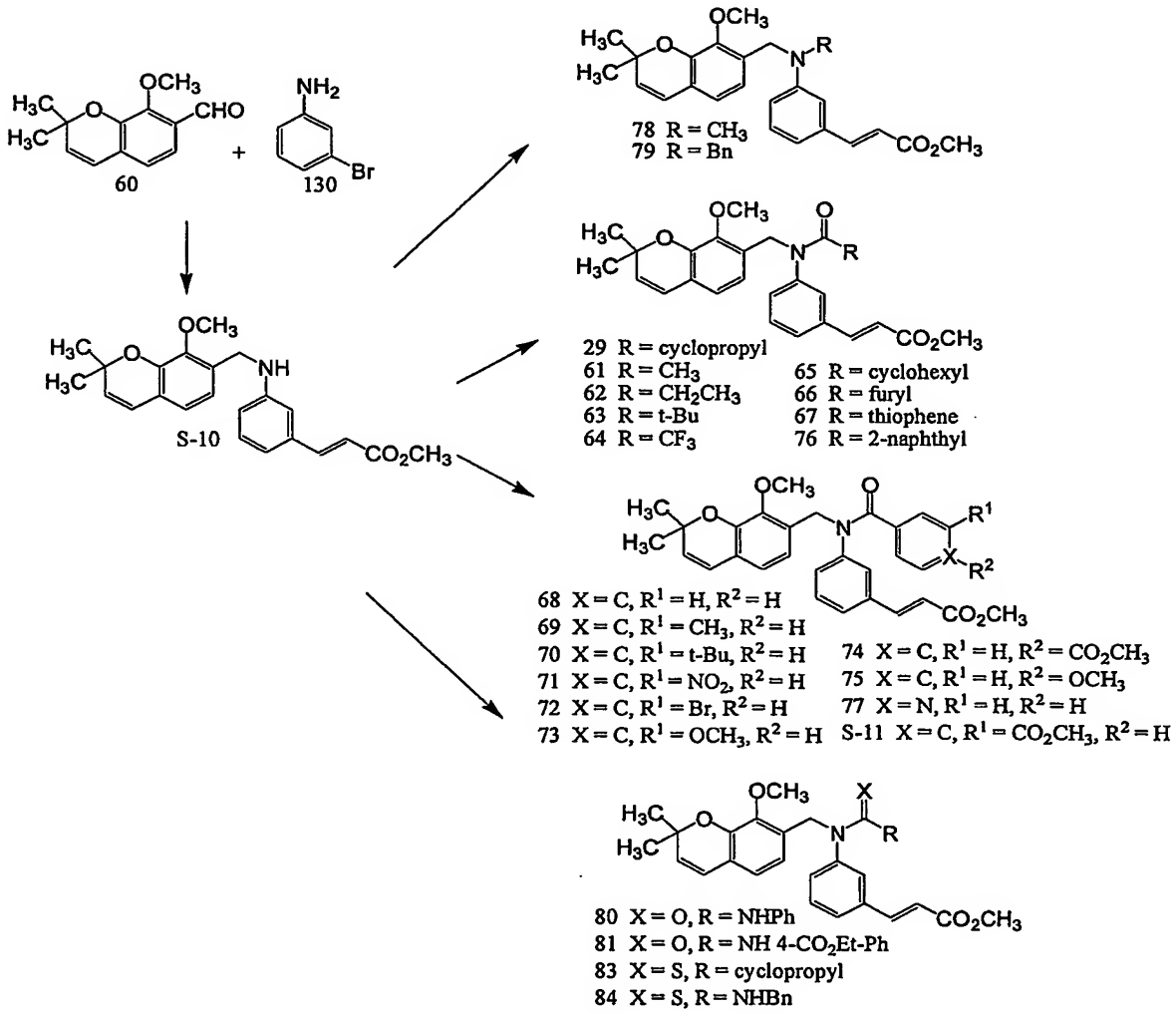
hydrolyzed (4.0 equiv.of LiOH, THF:H_2O (10:1), 25°C, 24 h. 88%) to the acid which is aminated by mixed anhydride formation followed by exposure to an amine, (1.2 equiv. of EtOC(O)Cl, 1.5 equiv. of Et_3N, 0.1 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 1 h., then 3.0 equiv. of NH_3, $MeNH_2$, $PhNH_2$ or $((CH_2)_5)N$, CH_2Cl_2, 25°C, 12 h., 85%-95%) to provide amides **37-40**. **29** also undergoes cyclopropanation (10.0 equiv. of CH_2N_2, 0.2 equiv. of $Pd(OAc)_2$, Et_2O, 25°C, 12 h., 95%) to afford compound **35**.

[0102] Representative procedures for the preparation of Region II modified compounds are shown in Examples 7 and 8.

Example 7

Preparation of Compounds 61-77, 80-84, S-10 and S-11

[0103] The strategy employed for the preparation of 61-77, 80-84, S-10 and S-11 is shown below.



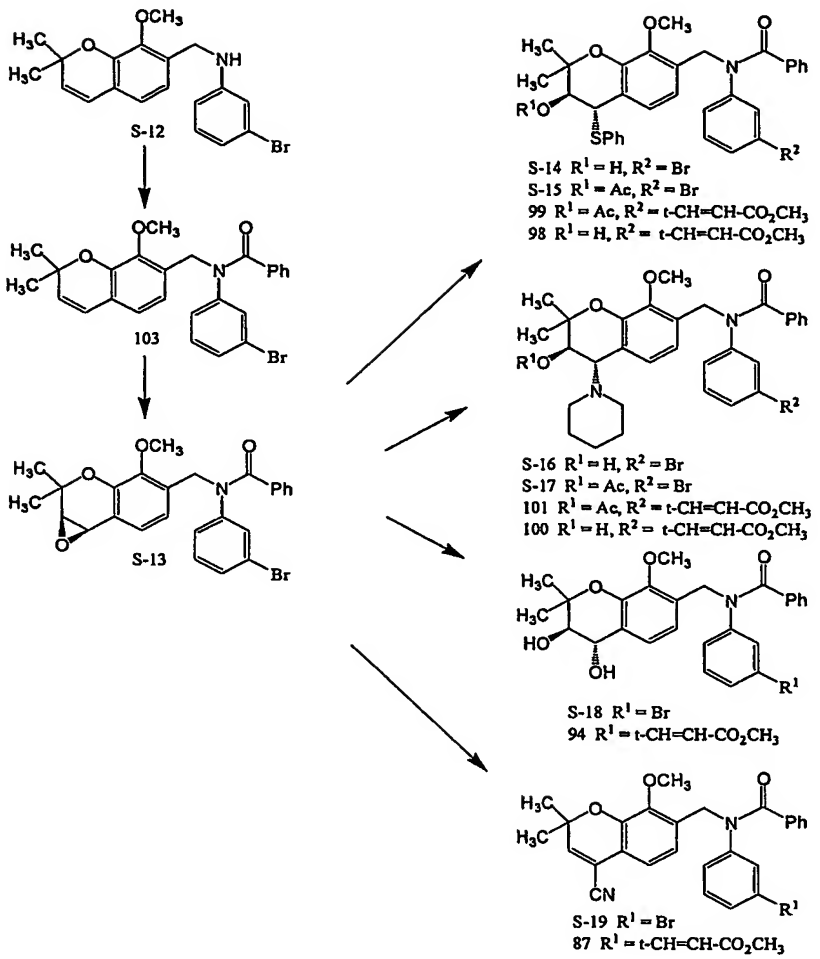
[0104] Thus, benzopyran 60 is reductively aminated (2.0 equiv. of 1-bromo-3-aminobenzene 130, THF, 70°C, 4 h., 70°C, then 2.0 equiv. of $NaCNBH_3$, 10% MeOH, 70°C,

4 h., 70%) and the intermediate amine coupled to methyl acrylate via a palladium mediated Heck reaction (1.5 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.5 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 24 h., 65%) to afford the amine **S-10**. **S-10** is N-alkylated (5.0 equiv. of NaH, 5.0 equiv. of PhBr, PhI or MeBr, EtOH, 80°C, 70%-85%) to provide compounds **78** and **79**, respectively. **S-10** is also N-acylated (5.0 equiv. of an acid chloride (RCOCl), 5.0 equiv. of Et_3N, 0.2 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 24 h. 55%-100%) to afford products **29**, **61-77** and **S-11**. **S-10** is further N-acylated (5.0 equiv. of an unsubstituted or substituted phenyl isocyanate RNCO, 5.0 equiv. of Et_3N, CH_2Cl_2, 25°C, 24 h.75%-85%) to provide urea products **80** and **81**, respectively. Finally, **S-10** amine is N-acylated (5.0 equiv. of a substituted phenyl isothiocyanate RNCS, 5.0 equiv. of Et_3N, CH_2Cl_2, 25°C, 24 h., 50%-70%) to afford the thiourea products **83** and **84**.

Example 8

Preparation of Compounds 87, 94, 98-101, 103 and S-13

[0105] The strategy employed for the preparation of **87**, **94**, **98-101**, **103** and **S-13** is shown below.



[0106] Thus, amine **S-12** is acylated (2.0 equiv. of benzoyl chloride, 2.0 equiv. of Et_3N, 0.2 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 24 h., 95%) to afford benzopyran amide **103**. **103** is oxidized (10 equiv. of DMDO, acetone, 0°C, 1 h., 100%) to provide epoxide **S-13** (100%). **S-13** undergoes ring opening (5.0 equiv. of PhSH, Amberlyst-15 catalyst, CH_2Cl_2, 25°C, 24 h., 95%) to afford the alcohol-sulfide compound **S-14**. **S-14** is acylated (2.0 equiv. of acetic anhydride, 2.0 equiv. of Et_3N, 0.2 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 24 h., 90%) to provide the acylated product, **S-15**. The acetate **S-15** and the alcohol **S-14** are coupled to methyl acrylate via a Heck reaction (2.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 70%-84%) to afford esters **98** and **99**, respectively. Epoxide **S-13** undergoes ring opening (5.0 equiv. of piperidine, CH_2Cl_2, 25°C, 24 h., 90%) to afford the alcohol-amino compound **S-16**. **S-16** is acylated (2.0 equiv. of

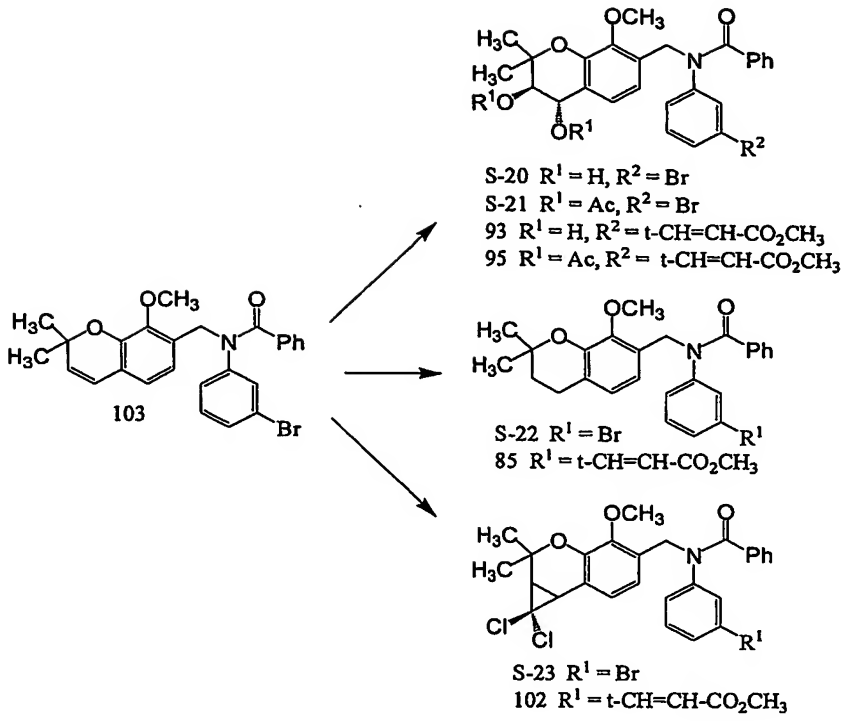
acetic anhydride, 2.0 equiv. of Et_3N, 0.2 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 24 h., 90%) to provide the acylated product, **S-17**. The acetate **S-17** and the alcohol **S-16** are coupled to methyl acrylate via a Heck reaction (2.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 70%-84%) to afford esters **100** and **101**, respectively. Similarly, epoxide **S-13** undergoes ring opening (5.0 equiv. of H_2O, Amberlyst-15 catalyst, THF, 25°C, 48 h., 95%) to afford the diol **S-18**. **S-18** is coupled to methyl acrylate via a Heck reaction (2.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 70%-84%) to provide ester **94**. Epoxide **S-13** also undergoes ring opening (2.0 equiv. of Et_2AlCN, CH_2Cl_2, O°C, 1 h., 83%) and elimination (40% KOH:MeOH (1:2), 25°C, 24 h., 90%) to afford the conjugated cyano compound **S-19**. **S-19** is coupled to methyl acrylate via a Heck reaction (2.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 70%-84%) to provide ester **87**.

[0107] Representative procedures for the preparation of Region III modified compounds are shown in Examples 9 to 17.

Example 9

Preparation of Compounds 85, 93, 95, 102, S-20, S-21, S-22 and S-23

[0108] The strategy employed for the preparation of **Compounds 85, 93, 95, 102, S-20, S-21, S-22** and **S-23** is shown below.

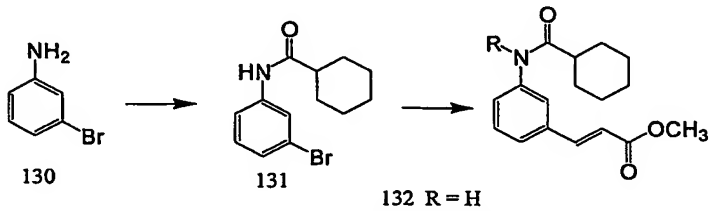


[0109] Thus, benzopyran amide **103** is oxidized (0.02 equiv. of OsO_4, 2.0 equiv. of NMO, acetone, H_2O (10:1), 25°C, 24 h., 85%) to afford diol **S-20**. **S-20** is diacylated (5.0 equiv. of acetic anhydride, 10.0 equiv. of Et_3N, 0.2 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 24 h., 90%) to provide the diacetate **S-21**. The diol **S-20** and the diacetate **S-21** are coupled to methyl acrylate via a Heck reaction (2.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 65%-80%) to afford esters **93** and **95**, respectively. Intermediate benzopyran amide **103** is reduced (10% Pd/C, EtOAc, 25°C, 0.5 h., 100%) to afford amide **S-22**. **S-22** is coupled to methyl acrylate via a Heck reaction (2.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 65%-80%) to provide ester **85**. Intermediate benzopyran amide **103** is also cyclopropanated (CHCl3, 50% NaOH, (7:1), adogen 464 catalyst, 25°C, 6 h., 85%) to afford compound **S-23**. **S-23** is coupled to methyl acrylate via a Heck reaction (2.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 24 h., 65%-80%) to provide ester **102**.

Example 10

Preparation of Compounds 110, 111, 114-118, 147 and 148

[0110] The strategy employed for the preparation of Compounds **110, 111, 114-118, 147** and **148** is shown below.



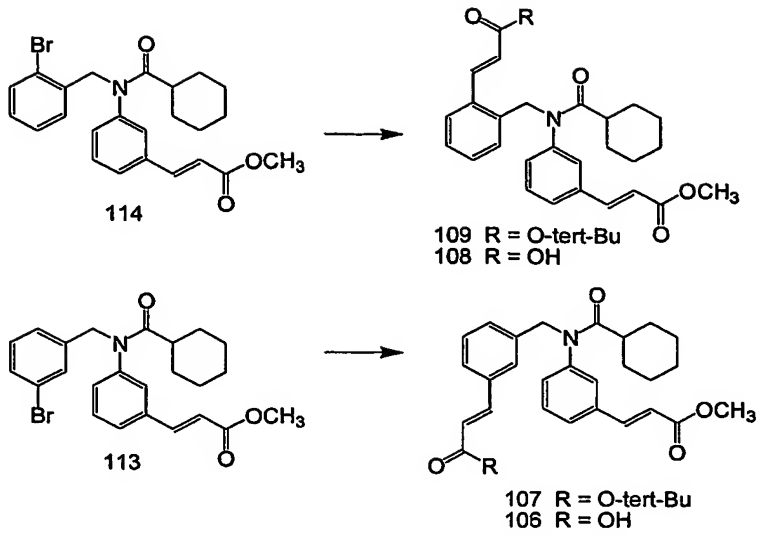
132 R = H
147 R = CH_3
110 R = benzyl
111 R = 2-naphthyl
114 R = 2-bromobenzyl
115 R = 3-bromobenzyl
116 R = 4-bromobenzyl
148 R = 4-tert-butylbenzyl
115 R = 3-methoxybenzyl
117 R = 3-(trifluoromethylbenzyl)
118 R = 3,5-dimethoxybenzyl

[0111] Thus, 3-bromo-aniline **130** is acylated (1.1 equiv. of $C_6H_{11}COCl$, 1.3 equiv. of Et_3N, 0.05 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 3 h., 95%) to afford amide **131**. **131** is coupled to methyl acrylate via a Heck reaction (4.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 12 h., 80%) to provide ester **132**. **132** is N-alkylated (1.1 equiv. of NaH, THF, O°C, 30 min., then 1.3 equiv. of benzyl bromides, THF, 2 h., 60%-90% where R-X = methyl iodide, benzyl bromide, 2-bromobenzyl bromide, 3-bromobenzyl bromide, 4-bromobenzyl bromide, 4-*tert*-butyl benzyl bromide, 3-methoxy benzyl bromide, 3,5-dimethoxy benzyl bromide, 3-(trifluoromethyl) benzyl bromide, 2-naphthyl benzyl bromide) to afford compounds **105, 110-112, 114-118** and **148**.

Example 11

Preparation of Compounds 106-109

[0112] The strategy employed for the preparation of Compounds **106-109** is shown below.

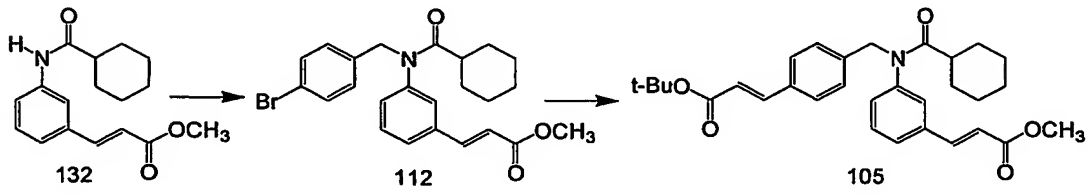


[0113] Thus, aryl bromides **114** and **115** are coupled to *tert*-butyl acrylate via a Heck reaction (4.0 equiv. of *tert*-butyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 12 h., 80%) to afford esters **109** and **110**, respectively. **109** and **110** are acidified (20% TFA in CH_2Cl_2, 25°C, 1 h., 95%) to provide acids **108** and **106**, respectively.

Example 12

Preparation of Compounds 105 and 112

[0114] The strategy employed for the preparation of Compounds **105 and 112** is shown below.

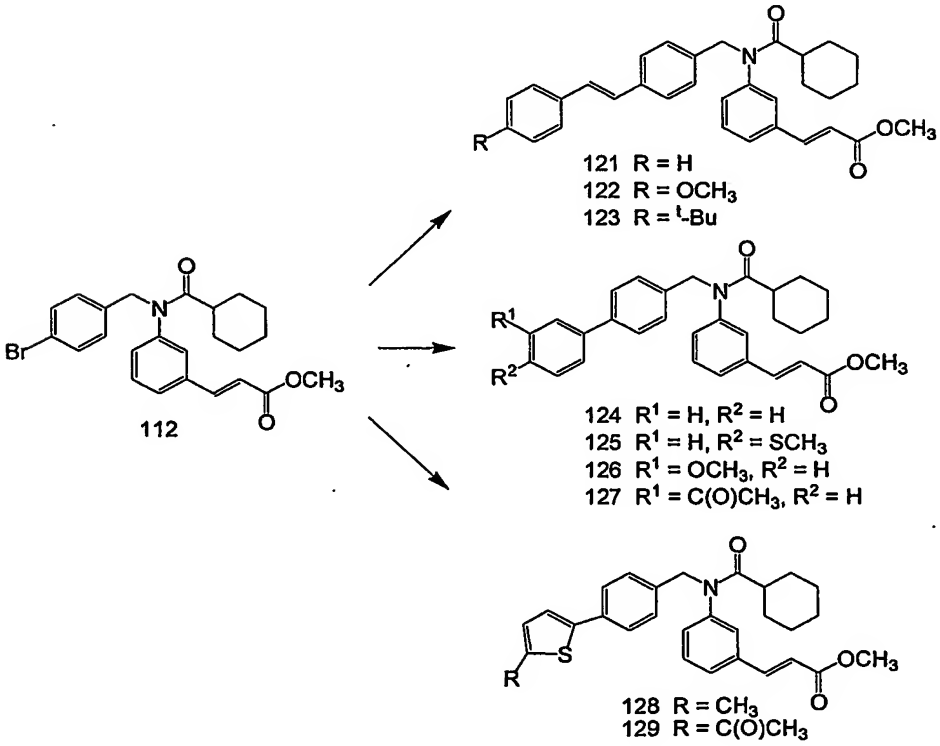


[0115] Thus, 3-bromo-aniline **130** is N-acylated (1.1 equiv. of $C_6H_{11}COCl$, 1.3 equiv. of Et_3N, 0.05 equiv. of 4-DMAP, CH_2Cl_2, 25oC, 3 h., 95%) to afford amide **131**. **131** is coupled to methyl acrylate via a Heck reaction (4.0 equiv. of methyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 12 h., 80%) to provide ester **132**. **132** is N-acylated (1.1 equiv. of para-bromoC_6H_4COCl, 1.3 equiv. of Et_3N, 0.05 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 3 h., 95%) to afford tertiary amide **112**. **112** is coupled to tert-butyl acrylate via a Heck reaction (4.0 equiv. of tert-butyl acrylate, 0.2 equiv. of $Pd_2(dba)_3$, 0.6 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 12 h., 80%) to provide diester **105**.

Example 13

Preparation of Compounds 121-129

[0116] The strategy employed for the preparation of Compounds **121-129** is shown below.

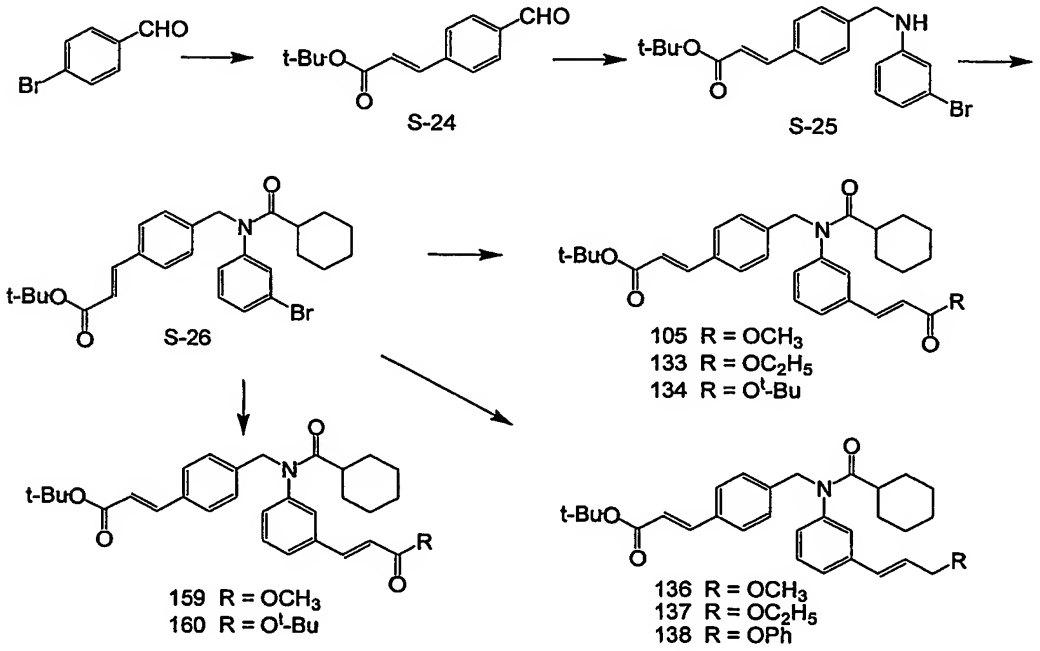


[0117] Thus, aryl bromide **112** is coupled to para-substituted styrene via a Heck reaction (4.0 equiv. of styrene, or para-methoxy styrene, or para *tert*-butyl styrene, 0.05 equiv. of $Pd_2(dba)_3$, 0.15 equiv. of P(*o*-tol)$_3$, 5.0 equiv. of Et_3N, DMF, 90°C, 12 h., 65%-80%) to afford esters **121, 122** and **123**, respectively. **112** is coupled to unsubstituted and substituted phenyl and thiophene via a Suzuki reaction (2.5 equiv. of boronic acid, 0.2 equiv. of $Pd(PPh_3)_4$, toluene:MeOH: 1M Na_2CO_3 (10:3:1), 80°C, 12 h., 60%-80%) to provide compounds **124-129.**

Example 14

Preparation of Compounds 105, 133, 134, 136-138, 159,160 and S-24

[0118] The strategy employed for the preparation of Compounds **105, 133, 134, 136-138, 159,160** and **S-24** is shown below.



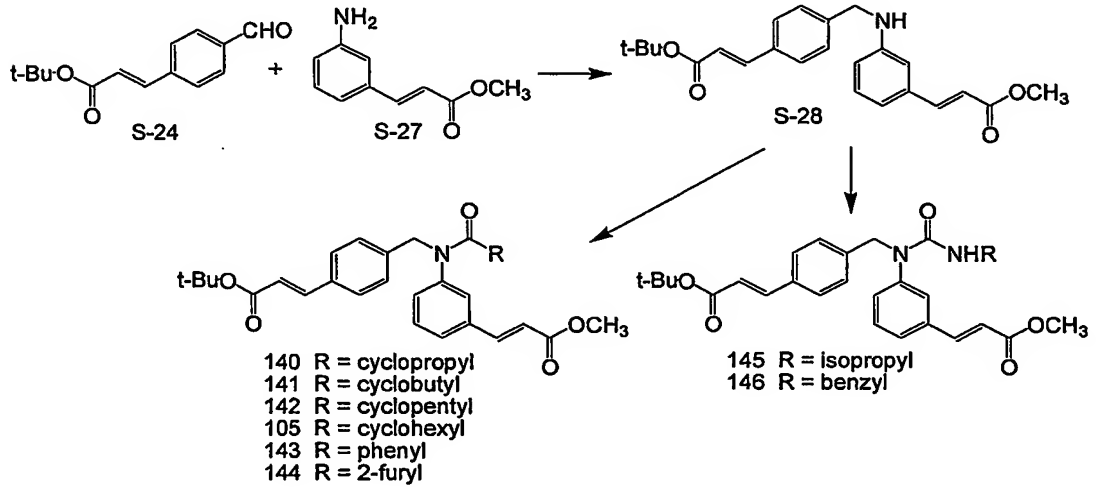
[0119] Thus, para-bromobenzaldehyde is coupled to *tert*-butyl acrylate via a Heck reaction (4.0 equiv. of *tert*-butyl acrylate, 5.0 equiv. of Et_3N, 0.05 equiv. of $Pd_2(dba)_3$, 0.15

equiv. of $P(o\text{-}tol)_3$, DMF, 90°C, 12 h., 85%) to afford aldehyde **S-24**. **S-24** is reductively aminated (1.5 equiv. of 3-bromoaniline, 0.05 equiv. of AcOH, MeOH, 25°C, 30 min., then 1.7 equiv. of $NaCNBH_3$, 1 h., 90%) to provide amine **S-25**, which is acylated (1.1 equiv. of $C_6H_{11}COCl$, 1.3 equiv. of Et_3N, 0.05 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 3 h., 90%) to afford aryl bromide **S-26**. **S-26** is coupled via a Heck reaction (4.0 equiv. of acrylate or 4.0 equiv. of allyl ether, 5.0 equiv. of Et_3N, 0.05 equiv. of $Pd_2(dba)_3$, 0.15 equiv. of $P(o\text{-}tol)_3$, DMF, 90°C, 12 h., 60%-85%) to provide compounds **105, 133, 134** and **136-138**.

Example 15

Preparation of Compounds 140-146 and S-28

[0120] The strategy employed for the preparation of Compounds **140-146** and **S-28** is shown below.



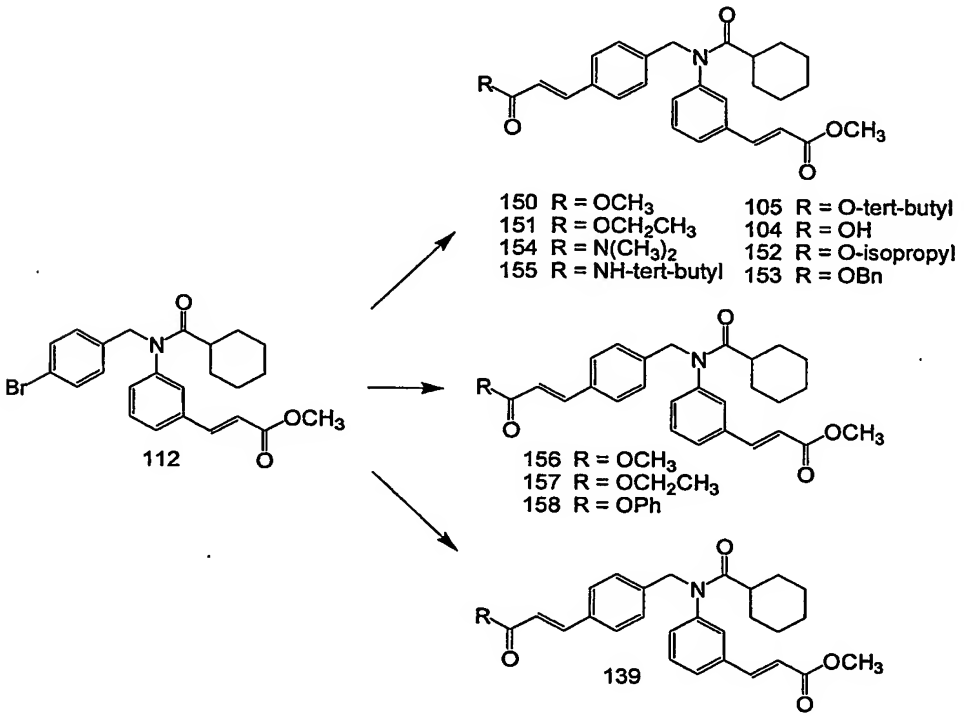
[0121] Thus, aldehyde **S-24** is coupled to amine **S-27** via a reductive amination (0.05 equiv. of AcOH, MeOH, 25°C, 30 min., then 1.2 equiv. of $NaCNBH_3$, 25°C, 1 h., 85%) to afford amine **S-28**. **S-28** is N-acylated (2.0 equiv. of acid chloride, 3.0 equiv. of Et_3N, 0.05 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 1 h., 80%-95%) to provide compounds **105** and **140-144**.

S-28 is also acylated (2.0 equiv. of isocyanate, 3.0 equiv. of Et_3N, 0.05 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 1 h., 60%-80%) to afford urea compounds **145** and **146**.

Example 16

Preparation of Compounds 104, 105, 139 and 150-158

[0122] The strategy employed for the preparation of Compounds **104, 105, 139** and **150-158** is shown below.



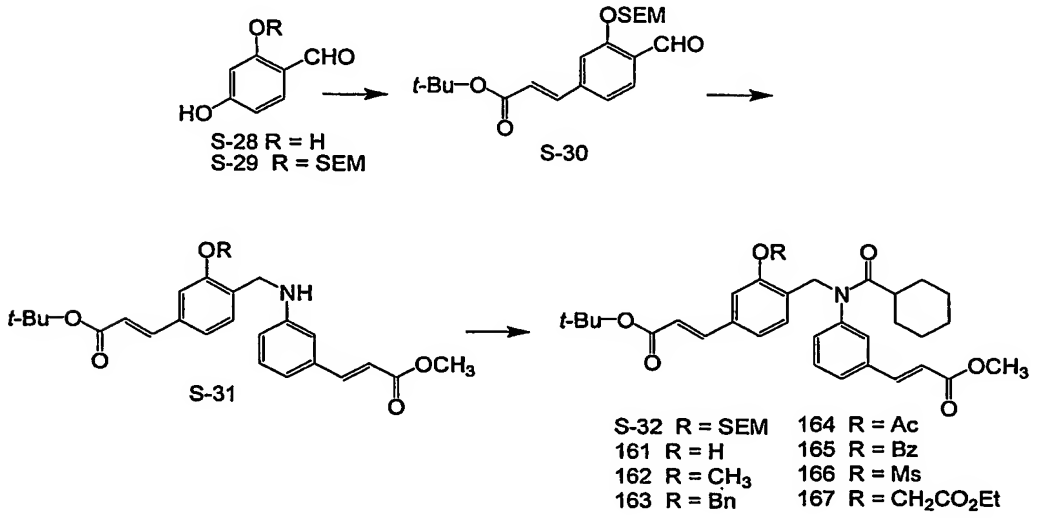
[0123] Thus, aryl bromide **112** is coupled to acrylates via a Heck reaction (4.0 equiv. of acrylate, 5.0 equiv. of Et_3N, 0.05 equiv. of $Pd_2(dba)_3$, 0.15 equiv. of P(o-tol)$_3$, DMF, 90°C, 12 h., 50%-80%) to afford compounds **105** and **150-155**. Ester **105** is hydrolyzed (20% TFA in CH_2Cl_2, 1 h., 25°C, 95%) to provide acid **104**. Acid **104** is esterified (1.2 equiv. of DCC, 10 equiv. of i-PrOH or BnOH, 0.2 equiv. of 4-DMAP, DMF, 25°C, 12 h., 60%) to afford compounds **152** and **153**, respectively. Aryl bromide **112** is coupled to alkenes via a Heck

reaction (4.0 equiv. of methyl vinyl ether, ethyl vinyl ether and phenyl vinyl ether, 5.0 equiv. of Et_3N, 0.05 equiv. of $Pd_2(dba)_3$, 0.15 equiv. of P(o-tol)$_3$, DMF, 90°C, 12 h., 50%-80%) to provide compounds **156** to **158**, respectively. Further, aryl bromide **112** is reduced (0.05 equiv. of 10% Pd/C, H_2 (1 atm.), EtOAc, 25oC, 30 min., 100%) to afford the saturated ester, which is coupled to *tert*-butyl acrylate via a Heck reaction (4.0 equiv. of *tert*-butyl acrylate, 5.0 equiv. of Et_3N, 0.05 equiv. of $Pd_2(dba)_3$, 0.15 equiv. of P(o-tol)$_3$, DMF, 90°C, 12 h., 35%-75%) to provide compound **139**.

Example 17

Preparation of Compounds 161-167 and S-29

[0124] The strategy employed for the preparation of Compounds **161-167** and **S-29** is shown below.



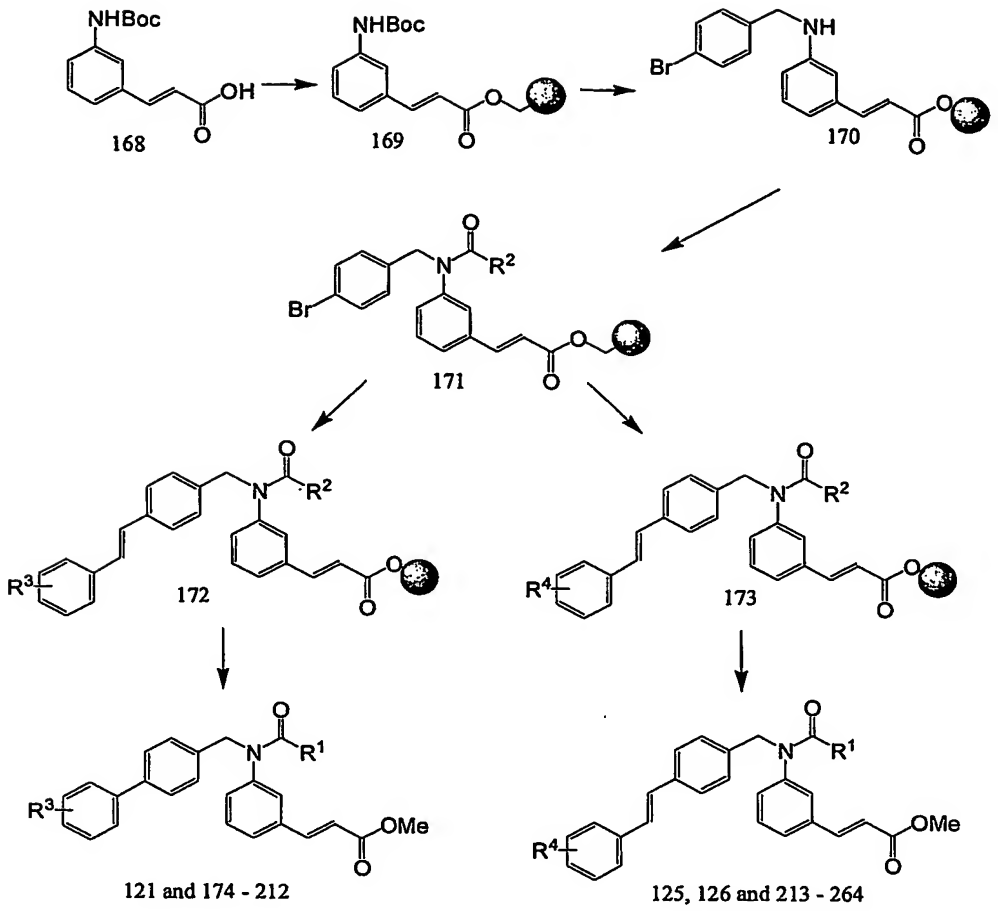
[0125] Thus, 2,4-dihydroxybenzaldehyde **S-28** is selectively monoprotected (1.0 equiv. of SEM-Cl, 1.2 equiv. of Et_3N, CH_2Cl_2, 25°C, 12 h., 75%) to afford the para hydroxyl compound **S-29**. **S-29** is O-alkylated (1.05 equiv. of Tf_2O, 1.2 equiv. of Et_3N, CH_2Cl_2, -78°C, 1 h., 95%) and the triflate is coupled to *tert*-butyl acrylate via a Heck reaction (4.0 equiv. of *tert*-butyl acrylate, 5.0 equiv. of Et_3N, 0.05 equiv. of $Pd_2(dba)_3$, 0.15 equiv. of P(o-tol)$_3$, DMF, 90°C, 12 h., 76%) to provide compound **S-30**. Aldehyde **S-30** is coupled to amine **S-**

27 via a reductive amination (1.2 equiv. of S-27, 0.05 equiv. of AcOH, MeOH, 25°C, 1 h., then 1.5 equiv. of $NaCNBH_3$, 2 h., 80%) to afford amine S-31. S-31 is N-acylated (1.2 equiv. of $C_6H_{11}COCl$, 1.5 equiv. of Et_3N, 0.05 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 4 h., 90%) to provide amide S-32. S-32 is deprotected (3.0 equiv. of BnBr, 5.0 equiv. of K_2CO_3, DMF, 80°C, 12 h., 65%) to afford alcohol **161**. **161** is alkylated with (3.0 equiv. of MeI, 5.0 equiv. of K_2CO_3, DMF, 80°C, 12 h., 90%) to provide methyl ether **162**; or with (3.0 equiv. of BnBr, 5.0 equiv. of K_2CO_3, DMF, 80°C, 12 h., 65%) to afford benzyl ether **163**, or acetylated with (3.0 equiv. of $BrCH_2COOEt$, 5.0 equiv. of K_2CO_3, DMF, 80°C, 12 h., 90%) to provide **167**. Alcohol **161** is O-alkylated (3.0 equiv. of AcCl, BzCl or MsCl, 5.0 equiv. of Et_3N, CH_2Cl_2, 2 h., 70%-90%) to provide compounds **164**, **165** and **166**, respectively.

Example 18

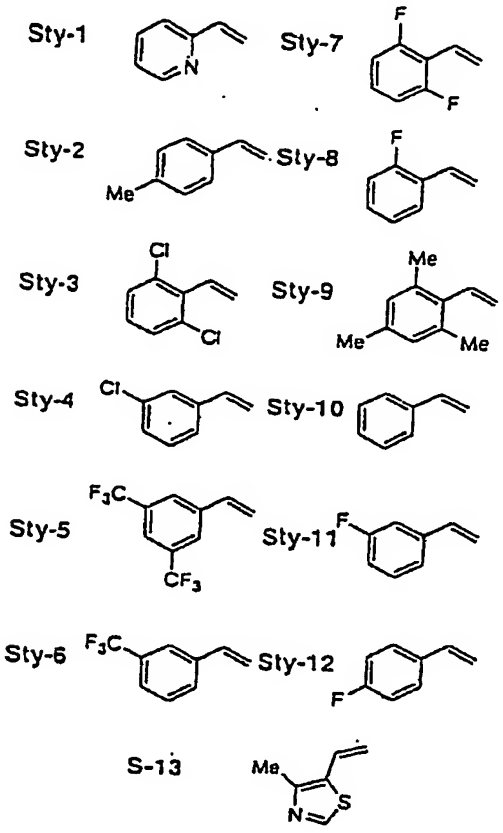
Preparation of Compounds 121, 125, 126 and 174-264

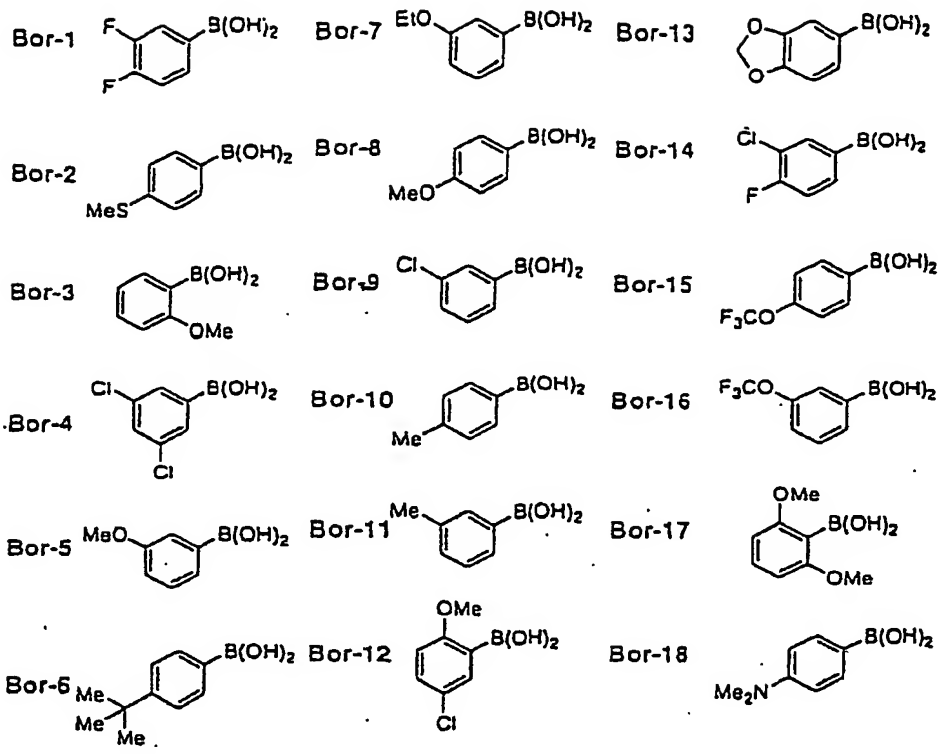
[0126] The strategy employed for the preparation of Compounds **121**, **125**, **126** and **174-264** is shown below.



[0127] Thus, Boc protected cinnamic acid **168** is immobilized on resin (1.0 equiv. of Merrifield Resin, (0.91 mmol/mg), 2.0 equiv. of Cs_2CO_3, 0.5 equiv. of TBAI, DMF, 55°C, 24 h.) to afford resin **169**. **169** is deprotected (20% TFA in CH_2Cl_2, 25°C, 1 h.) and the resultant resin-bound amine is reductively alkylated with 4-bromobenzaldehyde (10.0 equiv. of 4-aminobenzaldehyde, 0.05 equiv. of AcOH, THF:MeOH (2:1), 25°C, 1 h., then 8 equiv. of $NaCNBH_3$, THF:MeOH (2:1), 25°C, 2 h.) to provide amino resin **170**. **170** is acylated (for R^1COCl: 30 equiv. of R^1COCl, 40.0 equiv. of Et_3N, 1.0 equiv. of 4-DMAP, CH_2Cl_2, 25°C, 12 h., for R^1NCO, 30.0 equiv. of R^1NCO, 40.0 equiv. of Et_3N, 1.0 equiv. of 4-DMAP, DMF, 65°C, 60 h.) with one of three acyl groups to afford amide or urea resins **171**. The acylated resins (**171**) were subjected to either Heck coupling with thirteen substituted styrenes (as illustrated below; 8.0 equiv. of styrene, 10.0 equiv. of Et_3N, 0.5 equiv. of $Pd_2(dba)_3$, 1.5 equiv. of P(*o*-tol)$_3$, DMF, 90°C, 48 h.) or Suzuki coupling with eighteen boronic acids (as

illustrated below; 5.0 equiv. of boronic acid, 3.0 equiv. of Cs_2CO_3, 0.5 equiv. of $Pd(PPh_3)_4$, DMF, 90°C, 24 h.) to provide stilbene resins **172** and biaryl resins **173**, respectively.



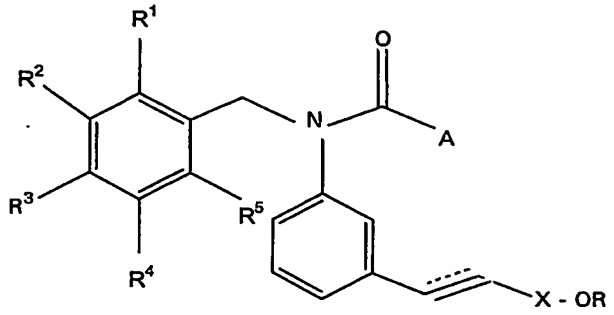


[0128] Hydrolysis of these resins (**172** and **173**) with base (10.0 equiv. of NaOMe, Et_2O:MeOH (10:1), 25°C, 20 min.) affords compounds **121, 125, 126** and **174-264**. Analysis of the library by LCMS after purification showed the average purity of these compounds to be > 95%.

[0129] It will be apparent to those skilled in the art that various changes may be made in the invention without departing from the spirit and scope thereof, and therefore, the invention encompasses embodiments in addition to those specifically disclosed in the specification, but only as indicated in the appended claims.

That which is claimed is:

1. A compound having the structure:



wherein:

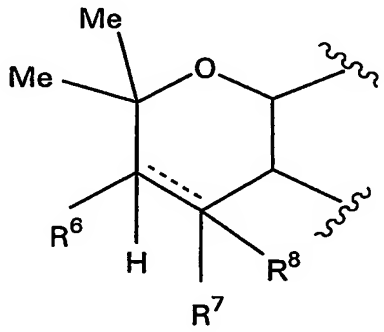
A is a C3 up to C8 branched chain alkyl or substituted alkyl group, a C3 up to C7 cycloalkyl or substituted cycloalkyl, an optionally substituted aryl or an optionally substituted heteroaryl,

X is –C(O)- or –CH_2-,

R is methyl or ethyl,

R^1 is H, hydroxy, alkoxy, benzoyloxy, mesityloxy, or –$OCH_2C(O)OC_2H_5$,

R^2 is H or R^2 can cooperate with R^3 to form a benzopyran, wherein the pyran ring has the structure:



wherein:

R^6 is not present if the pyran ring is unsaturated, or, if present, is selected from H, -OR, wherein R is alkyl or acyl, or R^6 can cooperate with R^7 to form a cyclic acetal, a cyclic ketal, or a cyclopropyl moiety, and

only one of R^7 and R^8 is present if the pyran ring is unsaturated, or R^7 and R^8 are independently H, carboxyl, cyano, hydroxy, alkoxy, thioalkyl, aryl, or R^7 and R^8 taken together comprise a carbonyl oxygen or an oxime nitrogen, or either R^7 or R^8 can cooperate with R^6 to form a cyclic acetal, a cyclic ketal, or a cyclopropyl moiety,

R^3 can cooperate with R^2 to form a benzopyran having the structure set forth above, or R^3 is alkenyl, optionally substituted aryl or heteroaryl, or optionally substituted arylalkenyl or heteroarylalkenyl,

R^4 is H or hydroxy, and

R^5 is H, hydroxy, alkoxy or aryloxy.

2. The compound of claim 1 wherein R^2 and R^3 cooperate to form a benzopyran.

3. The compound of claim 2 wherein A is cyclopropyl, X is -C(O)-, R^1 is methoxy, R^6 and R^7 are absent, and R^4, R^5 and R^8 are hydrogen.

4. The compound of claim 2 wherein A is cyclopropyl, X is $-CH_2-$, R^1 is methoxy, R^6 and R^7 are absent, and R^4, R^5 and R^8 are hydrogen.

5. The compound of claim 2 wherein A is cyclohexyl, X is -C(O)-, R^1 is methoxy, R^6 and R^7 are absent, and R^4, R^5 and R^8 are hydrogen.

6. The compound of claim 2 wherein A is phenyl, X is -C(O)-, R^1 is methoxy, R^6 and R^7 are absent, and R^4, R^5 and R^8 are hydrogen.

7. The compound of claim 2 wherein A is phenyl, X is -C(O)-, R^1 is methoxy, R^6 and R^7 cooperate to form a dichlorocyclopropyl ring, and R^4, R^5 and R^8 are hydrogen.

8. The compound of claim 2 wherein A is cyclohexyl, X is -C(O)-, R^1 is methoxy, R^6 and R^7 cooperate to form a dichlorocyclopropyl ring, and R^4, R^5 and R^8 are hydrogen.

9. The compound of claim 1 wherein R^3 is alkenyl.

10. The compound of claim 9 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-C(O)-O-tBu.

11. The compound of claim 1 wherein R^3 is optionally substituted aryl or heteroaryl.

12. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is phenyl.

13. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is p-thiomethyl-phenyl.

14. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is m-methoxy-phenyl.

15. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is m-acetyl-phenyl.

16. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is 5-methyl-2-thiophene-yl.

17. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is 5-acetyl-2-thiophene-yl.

18. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is 4-dimethylamino-phenyl.

19. The compound of claim 11 wherein A is isopropyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is 4-dimethylamino-phenyl.

20. The compound of claim 11 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is 2,3-(O-CH_2-O)-phenyl.

21. The compound of claim 11 wherein A is isopropyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is 2,3-(O-CH_2-O)-phenyl.

22. The compound of claim 1 wherein R^3 is or optionally substituted arylalkenyl or heteroarylalkenyl.

23. The compound of claim 22 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-phenyl.

24. The compound of claim 22 wherein A is isopropyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-phenyl.

25. The compound of claim 22 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-p-methoxy-phenyl.

26. The compound of claim 22 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-o-fluoro-phenyl.

27. The compound of claim 22 wherein A is isopropyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-o-fluoro-phenyl.

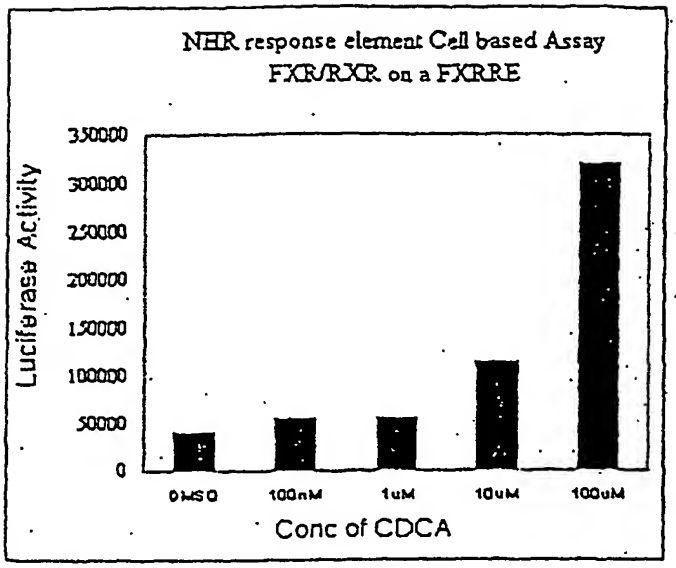
28. The compound of claim 22 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-m-fluoro-phenyl.

29. The compound of claim 22 wherein A is isopropyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-m-fluoro-phenyl.

30. The compound of claim 22 wherein A is cyclohexyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-p-fluoro-phenyl.

31. The compound of claim 22 wherein A is isopropyl, X is -C(O)-, R^1 R^2, R^4 and R^5 are hydrogen, and R^3 is –CH=CH-p-fluoro-phenyl.

32. A formulation comprising at least one compound according to claim 1 in a pharmaceutically acceptable carrier therefor.



FXR efficacy on a 384 well plate.

Figure 1